Sator OHDAKE: Isolation of Orizanin (Antineuritic Vitamin). Third Report. ... 71
Takeo NITO: On the organic Bases of the Flue-Cured Tobacco. ... 77
Kotaro NISHIDA: Biochemical Studies on "Sotetsu" the Japanese Sago Plant. I.—Changes of the General Composition of "Sotetsu"-seed during its Ripening. ... 78
Arao ITANO and Akira MATSUURA: Investigation on the Influence of Ultraviolet Rays on the Physiological Activities of Azotobacter.—I. On the Lethal Action of Ultra-violet Rays on Azotobacter Chroococcum. ... 81
Yoshikazu SAHASHI: On the Effect of Linoleic Acid and Yeast upon the Growth of Rats on High Fat Diet. ... 82
Umetaro SUZUKI, Waro NAKAHARA and Yoshikazu SAHASHI: The Occurrence of Vitamin E in soy Bean Oil. ... 87
Chikafumi ICHIKAWA: Über die Verteilung der Manganverbindung im Boden Japans, und ihre Beziehung auf die Fruchtbarkeit. ... 90
Masuzo SHIKATA and Koichiro KITAO: Researches on the Electric Boundary Layer Disturbance. Part IV.—The Effect of the Alternating Electric Field on the Permeability Potential of Membrane. ... 90
Masuzo SIKATA and Takeo KOHSAKA: Chemical Researches on the Pulp Woods in Karafuto. Part. V.—Cooking and Spinning Test of Karafuto Fir. ... 91
Eiichi SOMEKAWA: On the Production of Seborrhea in Rat by Feeding with Whale Oil. Part I. ... 93
Arao ITANO and Akira MATSUURA: Investigation on the Influence of Ultraviolet Rays on the Physiological Activities of Azotobacter.—II. On the Stimulation of Azotobacter Chroococcum by Ultra violet Rays. ... 101
Kiyotoki KATO: Studies on the Digestion of the Carbohydrates Contained in Mulberry Leaves by Silkworms. Part. II.—On the Digestibility and the Quantity of the Digestion of the Carbohydrates Contained in Mulberry Leaves in Different Stages of Growth. ... 102
Kenzi TAKEDA: Chemical Studies on "Bukuryo", Sclerotia of *Pachyma Hoelen* RUMPH. (IV).—Discussion of the Relation among, β-*Pachyman* and Related Compounds. ... 103

Sator OHDAKE: Isolation of Orizanin (Antineuritic Vitamin). Third Report. ... 71

Takeo NITO: On the organic Bases of the Flue-Cured Tobacco. ... 77

Kotaro NISHIDA: Biochemical Studies on "Sotetsu" the Japanese Sago Plant. I.—Changes of the General Composition of "Sotetsu"-seed during its Ripening. ... 78

Arao ITANO and Akira MATSUURA: Investigation on the Influence of Ultraviolet Rays on the Physiological Activities of Azotobacter.—I. On the Lethal Action of Ultra-violet Rays on Azotobacter Chroococcum. ... 81

Yoshikazu SAHASHI: On the Effect of Linoleic Acid and Yeast upon the Growth of Rats on High Fat Diet. ... 82

Umetaro SUZUKI, Waro NAKAHARA and Yoshikazu SAHASHI: The Occurrence of Vitamin E in soy Bean Oil. ... 87

Chikafumi ICHIKAWA: Über die Verteilung der Manganverbindung im Boden Japans, und ihre Beziehung auf die Fruchtbarkeit. ... 90

Masuzo SHIKATA and Koichiro KITAO: Researches on the Electric Boundary Layer Disturbance. Part IV.—The Effect of the Alternating Electric Field on the Permeability Potential of Membrane. ... 90

Masuzo SIKATA and Takeo KOHSAKA: Chemical Researches on the Pulp Woods in Karafuto. Part. V.—Cooking and Spinning Test of Karafuto Fir. ... 91

Eiichi SOMEKAWA: On the Production of Seborrhea in Rat by Feeding with Whale Oil. Part I. ... 93

Arao ITANO and Akira MATSUURA: Investigation on the Influence of Ultraviolet Rays on the Physiological Activities of Azotobacter.—II. On the Stimulation of Azotobacter Chroococcum by Ultra violet Rays. ... 101

Kiyotoki KATO: Studies on the Digestion of the Carbohydrates Contained in Mulberry Leaves by Silkworms. Part. II.—On the Digestibility and the Quantity of the Digestion of the Carbohydrates Contained in Mulberry Leaves in Different Stages of Growth. ... 102

Kenzi TAKEDA: Chemical Studies on "Bukuryo", Sclerotia of *Pachyma Hoelen* RUMPH. (IV).—Discussion of the Relation among, *β-Pachyman* and Related Compounds. ... 103

Kenzi TAKEDA: Chemical Studies on "Bukuryo", Sclerotia of *Pachyma Hoelen* RUMPH. (V).—Nutritive value of *Pachyman.* 103

C. Y. CHEN: On the Non-identity of Adenine and Vitamin B_4. 105

Koji OKANO, Tadao ABE and Iwao OHARA: On the Isolation of Apigenin from Manchurian Kaoliang (Andropogen sorghum, Brot). 109

Arao ITANO and Y. TUJI: Investigation on Agar. (Continued). Physicochemical Properties of Agar and their Influence on the Growth of Microorganisms. 111

Kazuo YAMAFUJI: Untersuchungen über die Enzyme von Bombyx mori L. VII. Mitteilung.—Über die Eierkatalase (1). 112

Kazuo YAMAFUJI: Untersuchungen über die Enzyme von Bombyx mori L. VIII. Mitteilung.—Über die Eierkatalase (2). 116

Kazuo YAMAFUJI: Untersuchungen über die Enzyme von Bombyx mori L. IX. Mitteilung.—Über die Proteasen und Amylase des Blutes (2). 119

Matsuo TOKUOKA and Hitoshi MOROOKA: Über die physico-chemische Eigenschaft des Harnstoffnitrats. 127

Waro NAKAHARA and Fumito INUKAI: Studies on Dietary Requirement for Lactation.—II. Presence of Lactation Promoting Substance in Liver Extract. 129

Fumito INUKAI, Tuneto HIGASHI and Waro NAKAHARA: On the Occurrence of Vitamin B_2 (=Vitamin G) in Sake and Sake-Kasu (Pressed Cake). 135

Michiyo TSUJIMURA: Isolation of Catechin, Tea Catechin II or Gallo-Catechin from Green Tea. 140

Matsuo TOKUOKA: Über die Hygroskopicitat der Düngemitteln. 148

Umetaro SUZUKI, Waro NAKAHARA and Yoshikazu SAHASHI: Further Evidence for the Occurrence of Vitamin E in Soy Bean Oil. 149

Kenzi TAKEDA: Chemical Studies on "Bukuryo" Sclerotia of *Pachyma Hoelen* RUMPH. (VI).—Hydrolytic Oxidation Products of *β-Pachyman* by Conc. Sulphuric Acid and Conc. Nitric Acid. 152

Hideo KATAGIRI and Kakuo KITAHARA: The Characteristics of the Lactic Acid Bacteria Isolated from Moto, Yeast Mashes for Saké Manufacture. Part I.—The Fermentation Products From Koji Extract. 153

Hideo KATAGIRI, Kakuo KITAHARA and Kanji FUKAMI: The Chara-

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Contents

Sogo TETSUMOTO: Sterilizing Action of Acids (V)—Sterilizing action of dibasic fatty acids on Putrifactive bacteria, Bac. typhosus and vib. cholerae. ... 1
Masami OKU: Bombilupeol, a Colorless Crystallizable Constituent of the Alcoholic Extract of the Domestic Cocoon. ... 9
Mitunori WADA: Über die Bildung des Harnstoffs aus Uraminosauren, Hydantoinen und aus Eiweisskörpern durch Einwirkung von Enzymen (Reduktasen) in neutraler Lösung. ... 11
Kazuo YAMAFUJI: Untersuchungen über die Enzyme von Bombyx mori L. IV. Mitteilung.—Über die Phenolasen des Blutes. ... 15
Kazuo YAMAFUJI: Untersuchungen über die Enzyme von Bombyx mori L. V. Mitteilung—Über die Saccharase und Maltase des Blutes. ... 19
Kunijiro TAKEUCHI: Synthesis of 5-Oxy-camphor. ... 25
Toyosaku MINAGAWA: Studies on Amyrosynthease. ... 33
Hisasi ARIYAMA: On the Glycogen Content in the Liver of Pigeons and Rats by B_1-Avitaminosis. ... 47
Kazuo YAMAFUJI: Untersuchungen über die Enzyme von Bombyx mori L. VI. Mitteilung. ... 57
Masayoshi OGAWA: Nutritive Value of Canavanin (amino acid) ... 61
Isamu TACHI: Researches on the Electrolytic Reduction Potentials of Organic Compounds. Part XVIII.—On the Electrolytic Reduction of Camphor, again. ... 62
Tomotsune TAIRA and Tsuneo MASUZIMA: Hochsiedende unverseifbare Substanz des Fuselöls (II. Mitteilung). ... 63
Yukitaro KISHI: Studies on the Proteins Contained in Mulberry Leaves. Part IV.—A Comparative Study of the Digestibility of the Proteins Contained in Mulberry Leaves. ... 67
Yukitaro KISHI and Yonekichi YOKOTA: Studies on the Proteins Contained in Mulberry Leaves. Part V.—On the Quantitative Changes of All the Proteins (Considered Collectively) Contained in a Given Quantity of Fresh Mulberry Leaves, as Considered with Relation to the Growth of these Leaves. ... 69

Sator **OHDAKE**: Isolation of Orizanin (Antineuritic Vitamin). Third Report. ... 71

Takeo **NITO**: On the organic Bases of the Flue-Cured Tobacco. ... 77

Kotaro **NISHIDA**: Biochemical Studies on "Sotetsu" the Japanese Sago Plant. I.—Changes of the General Composition of "Sotetsu"-seed during its Ripening. ... 78

Arao **ITANO** and Akira **MATSUURA**: Investigation on the Influence of Ultraviolet Rays on the Physiological Activities of Azotobacter.—I. On the Lethal Action of Ultra-violet Rays on Azotobacter Chroococcum. ... 81

Yoshikazu **SAHASHI**: On the Effect of Linoleic Acid and Yeast upon the Growth of Rats on High Fat Diet. ... 82

Umetaro **SUZUKI**, Waro **NAKAHARA** and Yoshikazu **SAHASHI**: The Occurrence of Vitamin E in soy Bean Oil. ... 87

Chikafumi **ICHIKAWA**: Über die Verteilung der Manganverbindung im Boden Japans, und ihre Beziehung auf die Fruchtbarkeit. ... 90

Masuzo **SHIKATA** and Koichiro **KITAO**: Researches on the Electric Boundary Layer Disturbance. Part IV.—The Effect of the Alternating Electric Field on the Permeability Potential of Membrane. ... 90

Masuzo **SIKATA** and Takeo **KOHSAKA**: Chemical Researches on the Pulp Woods in Karafuto. Part. V.—Cooking and Spinning Test of Karafuto Fir. ... 91

Eiichi **SOMEKAWA**: On the Production of Seborrhea in Rat by Feeding with Whale Oil. Part I. ... 93

Arao **ITANO** and Akira **MATSUURA**: Investigation on the Influence of Ultraviolet Rays on the Physiological Activities of Azotobacter.—II. On the Stimulation of Azotobacter Chroococcum by Ultra violet Rays. ... 101

Kiyotoki **KATO**: Studies on the Digestion of the Carbohydrates Contained in Mulberry Leaves by Silkworms. Part. II.—On the Digestibility and the Quantity of the Digestion of the Carbohydrates Contained in Mulberry Leaves in Different Stages of Growth. ... 102

Kenzi **TAKEDA**: Chemical Studies on "Bukuryo", Sclerotia of *Pachyma Hoelen* RUMPH. (IV).—Discussion of the Relation among, *β-Pachyman* and Related Compounds. ... 103

Kenzi TAKEDA: Chemical Studies on "Bukuryo", Sclerotia of *Pachyma Hoelen* RUMPH. (V).—Nutritive value of *Pachyman.* 103

C. Y. CHEN: On the Non-identity of Adenine and Vitamin B_4. 105

Koji OKANO, Tadao ABE and Iwao OHARA: On the Isolation of Apigenin from Manchurian Kaoliang (Andropogen sorghum, Brot). 109

Arao ITANO and Y. TUJI: Investigation on Agar. (Continued). Physicochemical Properties of Agar and their Influence on the Growth of Microorganisms. 111

Kazuo YAMAFUJI: Untersuchungen über die Enzyme von Bombyx mori L. VII. Mitteilung.—Über die Eierkatalase (1). 112

Kazuo YAMAFUJI: Untersuchungen über die Enzyme von Bombyx mori L. VIII. Mitteilung.—Über die Eierkatalase (2). 116

Kazuo YAMAFUJI: Untersuchungen über die Enzyme von Bombyx mori L. IX. Mitteilung.—Über die Proteasen und Amylase des Blutes (2). 119

Matsuo TOKUOKA and Hitoshi MOROOKA: Über die physico-chemische Eigenschaft des Harnstoffnitrats. 127

Waro NAKAHARA and Fumito INUKAI: Studies on Dietary Requirement for Lactation.—II. Presence of Lactation Promoting Substance in Liver Extract. 129

Fumito INUKAI, Tuneto HIGASHI and Waro NAKAHARA: On the Occurrence of Vitamin B_2 (=Vitamin G) in Sake and Sake-Kasu (Pressed Cake). 135

Michiyo TSUJIMURA: Isolation of Catechin, Tea Catechin II or Gallo-Catechin from Green Tea. 140

Matsuo TOKUOKA: Über die Hygroskopicität der Düngemitteln. 148

Umetaro SUZUKI, Waro NAKAHARA and Yoshikazu SAHASHI: Further Evidence for the Occurrence of Vitamin E in Soy Bean Oil. 149

Kenzi TAKEDA: Chemical Studies on "Bukuryo" Sclerotia of *Pachyma Hoelen* RUMPH. (VI).—Hydrolytic Oxidation Products of *β-Pachyman* by Conc. Sulphuric Acid and Conc. Nitric Acid. 152

Hideo KATAGIRI and Kakuo KITAHARA: The Characteristics of the Lactic Acid Bacteria Isolated from Moto, Yeast Mashes for Saké Manufacture. Part I.—The Fermentation Products From Koji Extract. 153

Hideo KATAGIRI, Kakuo KITAHARA and Kanji FUKAMI: The Characteristics of the Lactic Acid Bacteria Isolated from Moto, Yeast Mashes for Saké Manufacture. Part II.—Obserbation of Fermentable Sugars and Some Cultural Conditions. 154

Hideo KATAGIRI, Kakuo KITAHARA, Kanji FUKAMI and Minami SUGASE: The Characteristics of the Lactic Acid Bacteria Isolated from Moto, Yeast Mashes for Saké Manufacture. Part III.—The Fermentation Products from Pentoses and Hexoses. 155

Hideo KATAGIRI, Kakuo KITAHARA and Kanji FUKAMI: The Characteristics of the Lactic Acid Bacteria Isolated from Moto, Yeast Mashes for Saké Manufacture. Part IV.—The Classification of the Lactic Acid Bacteria. 156

Masami OKU: On the Natural Pigments of Raw Silk Fibre of the Domestic Cocoon (Part. VII).—On the Pigments of the Green Cocoon (Bombyx mori. var Seihaku). 158

Kenji TAKEDA: Chemical Studies on "Bukuryo" Sclerotia of *Pachyma Hoelen* RUMPH (VII).—The Production of Tetracetyl Glucuronic Acid by Acetolysis of *β-Pachyman* in the Presene of Perchloric Acid. 160

Kitsuji NISHIDA, Hideo HASHIMA und Takuichi FUKAMI: Polysaccharide (VIII).—Ein neues Kohlenhydrate "Xyloglukuronid" aus Kadsura japonica Dun. 162

Masami OKU: On the Natural Pigments of Raw Silk Fibre of the Domestic Cocoon (Part VIII).—Isoquercitrin from Mulberry Leaves. ... 164

Sogo TETSUMOTO: Sterilizing Power ef Fruits Juice and Condiments on Bac. Typhosus and Vib. Cholerae. 166

Waro NAKAHARA, Fumito INUKAI and Shu KATO: Liberation of Vitamin B_2 (Vitamin G) Adsorbed on Acid Earth by the Action of Pancreatin. 176

Suttekiti MARUYAMA: Isolation of Vitamin C from Vegetables and the Relations between Vitamin C and Ascorbic Acid. 184

Matuo TOKUOKA: Kationenumtausch an den Tonen (I. Mitteilung). 192

Kotaro NISHIDA and Aritomo YAMADA: Biochemical Studies on "Sotetsu", the Japanese Sago Plant. II.—On the Chemical Constituents, especially the Sex Differences of "Sotetsu"-Stems. 193

Sterilizing Action of Acids. (V).

Sterilizing action of dibasic fatty acids on Putrifactive bacteria, Bac. typhosus and Vib. cholerae.

By

Sogo TETSUMOTO

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Contents

(1) Reagents.
(2) Sterilizing action at the same molecular concentration.
(3) Sterilizing action of anions.
(4) Comparison of sterilizing action between dibasic and monobasic fatty acids of the same molecular concentration.
(5) Comparison of sterilizing action between dibasic and monobasic fatty acids at the same pH

Details of the sterilizing action of monobasic fatty acids are as reported previously.(1), (2)

To know the sterilizing action of dibasic fatty acids on Staphylococcus pyogenes aureus, Proteus vulgaris, Hauser, Bac. typhosus and Vib. cholerae, I used the following acids.

(1) Reagents.

Table I.

Acid	Number of C atom	Rational formulae	Molecular weight	pH		
				N/10	N/100	N/1000
Oxalic	C_2	$\begin{matrix}CO_2H\\ \mid\\ CO_2H\end{matrix} \cdot 2H_2O$	126 048	1 52	2.09	3.09
Malonic	C_3	$CH_2\Big\langle\begin{matrix}CO_2H\\ CO_2H\end{matrix}$	104.048	2 04	2.64	3.2
Succinic	C_4	$(CH_2)_2\Big\langle\begin{matrix}CO_2H\\ CO_2H\end{matrix}$	118.048	2 6	3.1	3.6
Glutaric	C_5	$(CH_2)_3\Big\langle\begin{matrix}CO_2H\\ CO_2H\end{matrix}$	132.089	2.66	3.2	3.66
Adipinic	C_6	$(CH_2)_4\Big\langle\begin{matrix}CO_2H\\ CO_2H\end{matrix}$	146.110	2 72	3.22	3 72
Pimelinic	C_7	$(CH_2)_5\Big\langle\begin{matrix}CO_2H\\ CO_2H\end{matrix}$				
Suberinic	C_8	$(CH_2)_6\Big\langle\begin{matrix}CO_2H\\ CO_2H\end{matrix}$	176.152	——	3.26	3.76
Azelainic	C_9	$(CH_2)_7\Big\langle\begin{matrix}CO_2H\\ CO_2H\end{matrix}$	188.173	——	3.30	3.80
Sebacinic	C_{10}	$(CH_2)_8\Big\langle\begin{matrix}CO_2H\\ CO_2H\end{matrix}$	202.194	——	——	3.78

(2) Sterilizing action at the same molecular concentration.

I made *N*/10, *N*/100 and *N*/1000 of aquoeus soltion by acids mentioned previously in Table I.

Results are mentioned in the following table.

Table II.

(1) Sterilizing action at *N*/10.

Acid	pH	Surviving period																	
		Staph c pyogen.						Prot vulgar , H					Bac. typhos.					Vib chol.	
		m 2.5	5	10	20	h 2	3	m 1	2 5	5	60	90	m 1	5	10	90	h 2	m 1	2 5
Oxalic	2 5	+	−	−	−	−	−	−	−	−	−	−	±	−	−	−	−	−	−
Malonic	2 0	+	+	±	−	−	−	+	+	−	−	−	+	+	−	−	−	−	−
Succinic	2.9	+	+	+	+	+	−	+	+	+	+	−	+	+	+	+	−	±	−
Glutaric	2 7	+	+	+	+	+	−	+	+	+	+	−	+	+	+	+	−	±	−
Adipinic	〃	+	+	+	+	±	−	+	+	+	+	−	+	+	+	±	−	±	−
Control		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

(2) Sterilizing action at *N*/100.

Acid	pH	Surviving period																													
		Staph c pyog							Prot valgar H								Bac typhosus								Vib choler						
		m 45	60	h 6	9	12	24	36	m 15	20	30	60	h 3	9	12	18	m 20	30	45	h 2	3	6	12	24	m 1	2 5	5	10	20	30	60
Oxalic	2.1	±	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	+	−	−	−	−	−
Malon.	2.6	+	+	−	−	−	−	−	+	+	+	−	−	−	−	−	+	+	+	−	−	−	−	−	+	+	+	−	−	−	−
Succin.	3.1	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−
Glutar.	3 2	+	+	+	+	+	+	−	+	+	+	+	+	+	±	−	+	+	+	+	+	+	+	−	+	+	+	+	+	−	−
Adipin	〃	+	+	+	+	+	±	−	+	+	+	+	+	+	−	−	+	+	+	+	+	+	±	−	+	+	+	+	±	−	−
Suberin	3.3	+	+	+	+	−	−	−	+	+	+	+	−	−	−	−	+	+	+	+	+	−	−	−	+	+	+	+	−	−	−
Azelain	〃	+	+	±	−	−	−	−	+	+	+	−	−	−	−	−	+	+	+	−	−	−	−	−	+	+	±	−	−	−	−
Control		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

(3) Sterilizing action at *N*/1000.

Acid	pH	Surviving period																											
		Staph. c pyogen.							Prot. vulgar , H.								Bac. typhosus								Vib. choler.					
		h 6	9	12	24	36	48	72	h 2	3	6	9	12	24	36	48	h 3	6	9	12	24	36	48	60	m 10	20	30	45	60	90
Oxal.	3.1	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−	−

Malon.	3.2	+	+	±	−	−	−	−	+	+	+	−	−	−	−	−	−	+	+	+	−	−	−	−	−	+	+	−	−	−
Succin.	3.6	+	+	+	+	+	+	−	+	+	+	+	+	+	±	−	+	+	+	+	+	+	±	−	+	+	+	+	+	+
Glut.	3.7	+	+	+	+	+	±	−	+	+	+	+	+	+	−	−	+	+	+	+	+	+	−	−	+	+	+	+	+	+
Adip.	"	+	+	+	+	+	−	−	+	+	+	+	+	−	−	−	+	+	+	+	+	±	−	−	+	+	+	+	+	+
Suber.	3.8	+	+	+	+	−	−	−	+	+	+	±	−	−	−	−	+	+	+	+	+	−	−	−	+	+	+	+	+	−
Azel.	"	+	+	+	−	−	−	−	+	+	+	−	−	−	−	−	+	+	+	−	−	−	−	−	+	+	+	+	−	−
Sebac.	"	+	−	−	−	−	−	−	+	±	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	±	−	−	−	−
Control		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

From the results noted in Table II (1), (2), (3) we find the following facts.

At the same molecular concentration of dibasic fatty acids, oxalic acid (C_2) is the strongest, malonic acid (C_3) and sebacinic acid (C_{10}) follow to this respectively as concerning the sterilizing action.

According to the increase of the number of C atom from C_3 acid, the sterilizing power greatly decrease. Succinic acid (C_4) is the weakest of all among dibasic fatty acids. From adipinic acid (C_6) according to the increase of the number of C atom, the solubility of acids to water greatly diminishes, but the sterilizing action increases gradually. On this respect, the fact resembles the sterilizing action of monobasic fatty acids.(1)

Notice: *N*/10 of suberinic acid, azelainic acid and sebacinic acid, and *N*/100 of sebacinic acid are not made because each acid insoluble in water at the such concentration, so I did not study.

(3) Sterilizing action of anions of dibasic fatty acids.

To know the sterilizing action of anions of dibasic fatty acids I made aqueous solution of Na, Ca and NH_4 salt having the same anion of dibasic fatty acids. Concentration of salts of C_2 to C_6 acid are *N*/100, and those of C_8 to C_{10} acids are *N*/1000 respectively.

Results are as shown in table III (1), (2) and (3).

Table III.—Sterilizing action of anions of dibasic fatty acids.

(1) Sterilizing action of Na salts.

Na-	Concent Nor.	Surviving period															
		Staph. pyogen				Prot vulgar.				Bac. typhos					Vib. choler.			
		6d	7	8	9	4d	5	6	7	4d	5	6	7	8	3h	6	9	12
Oxalate	1/100	+	±	−	−	+	−	−	−	+	±	−	−	−	+	+	±	−
Malonate	"	+	+	−	−	+	+	−	−	+	+	−	−	−	+	+	+	−
Succinate	"	+	+	+	−	+	+	±	−	+	+	+	±	−	+	+	+	−
Glutarate	"	+	+	+	−	+	+	±	−	+	+	+	−	−	+	+	+	−
Adipinate	"	+	+	±	−	+	±	−	−	+	+	+	−	−	+	+	+	−

(2) Results at $N/1000$ and saturated aqueous solution.

Number of C	Acid	pH	Surviving period							
			Staph. pyogen. (m)		Prot. vulgar. (m)		Bac. typhos. (m)		Vib. choler. (m)	
C_2	Acetic	3.9	2160	±	720	+	1440	+	60	+
	Oxalic	3.1	360	+	120	+	180	+	10	+
C_3	Propionic	3.9	2160	±	720	+	1440	+	60	+
	Acrylic	3.6	720	+	540	+	720	±	45	±
	Malonic	3.2	720	±	360	+	540	+	20	+
C_4	Butyric	3.9	2160	±	720	+	1440	+	60	+
	Isobutyric	〃	2880	+	1440	±	2160	+	90	+
	Crotonic	3.8	1440	+	720	+	1440	±	60	+
	Succinic	3.6	2880	+	2160	+	2880	±	90	+
C_5	Valeric	3.9	1440	+	720	+	1440	±	45	±
	Isovaleric	〃	2880	±	1440	±	2160	+	60	+
	Glutaric	3.7	2880	±	1440	+	2160	+	90	+
C_6	Caproic	3.9	1440	±	540	±	540	±	10	+
	Isocaproic	〃	2160	+	720	±	720	+	30	±
	Adipinic	3.7	〃	+	720	+	2160	±	90	±
C_8	Caplylic	4.8	20	±	2.5	±	5	±	1	−
	Suberinic	3.8	1440	±	540	±	1440	+	60	+
C_9	Perargonic	4.8	20	±	2.5	±	5	±	1	−
	Azelainic	3.8	720	+	360	+	540	+	45	+
C_{10}	Capric	4.8	20	±	2.5	±	5	±	1	−
	Sebacinic	3.8	360	+	180	±	180	+	20	+

Caprylic, pelargonic and capric acids are examined at saturated solution at 20°C.

From the results noted in table 4, we find the following facts. If we compare the sterilizing action at the same molecular concentration of acids having the same number of C atom, dibasic fatty acids are strongest, unsaturated monobasic fatty acids are next, and saturated monobasic fatty acids are weakest.

The fact is chiefly due to pH. At C_4 acids, unsaturated monobasic fatty acid is the strongest, saturated acid is the next and dibasic fatty acid is the weakest concerning the sterilization. From C_5 acid, according to the increase of the number of C atom, the sterilizing action of monobasic fatty acids are stronger than those of dibasic fatty acids. pH of monobasic fatty acids is higher than that of dibasic fatty acids, and that the sterilizing action of monobasic fatty acids are stronger than dibasic acids. The cause is due to the effect of undissociated molecule of each acids.

(5) Comparison of sterilizing action between dibasic and monobasic fatty acids at the same pH.

To find the sterilizing action at the same pH of dibasic fatty acids, and to compare the sterilizing action at the same pH of monobasic fatty acids, I made solution of pH 3.0 with C_2 to C_5 acids, pH 4.0 with C_2 to C_6 acids and pH 4.8 with C_8 to C_{10} acids, and compared them at the same number of C atom. Results are noted in table V (1), (2).

Table V.

(1) Comparison at pH 3.0.

Number of C	Acid	Normal	Surviving period							
			Staph. pyogen. (m)		Prot vulgar. (m)		Bac. typhos. (m)		Vib. choler. (m)	
C_2	Acetic	1/20	120	+	45	+	90	+	1	+
	Oxalic	1/900	360	+	120	+	180	±	10	+
C_3	Propion.	1/20	120	+	45	+	90	+	1	+
	Acrylic	〃	360	+	90	±	180	±	5	+
	Malonic	1/800	〃	+	180	±	〃	±	10	+
C_4	Butyric	1/20	120	+	45	+	90	+	1	+
	Isobutyr.	〃	180	+	60	+	120	+	2.5	+
	Croton.	1/40	360	±	90	±	〃	+	5	±
	Succinic	1/80	1440	±	540	+	720	±	20	+
C_5	Valeric	1/20	120	+	45	+	90	+	1	−
	Isovaler.	〃	180	+	60	+	120	+	2.5	+
	Glutaric	1/60	720	+	360	+	540	+	15	+

(2) Comparison at pH 4.0 and pH 4.8.

Number of C	Acid	Concent. Nor.	Surviving period							
			Staph. pyogen (m)		Prot. vulgar. (m)		Bac. typhos. (m)		Vib. choler. (m)	
C_2	Acetic	1/2000	2160	+	720	+	1440	+	60	+
	Oxalic	1/9000	5760	+	2160	+	2880	+	120	+
C_3	Propion.	1/2000	2160	+	720	+	1440	+	60	+
	Acrylic	1/8000	4320	+	2160	+	2880	+	120	+
	Maronic	1/8000	〃	+	〃	+	〃	+	90	+
C_4	Butyric	1/2000	2160	+	720	+	1440	+	60	+
	Isobutyr.	〃	2880	±	1440	+	2160	+	90	+
	Croton.	1/4000	4320	+	2160	±	2880	±	〃	+
	Succinic	1/8000	8640	+	4320	+	5760	±	180	±

C_5	Valeric	1/2000	2160	+	1440	+	1440	+	60	+
	Isovaler.	〃	2880	±	2160	±	2160	+	90	+
	Glutur.	1/6000	7200	±	2880	+	4320	±	120	+
C_6	Caproic	1/2000	1440	+	540	+	720	±	2.5	+
	Isocaproic	1/2000	2160	+	720	+	1440	+	60	+
	Adipin.	1/6000	5760	+	2160	+	4320	±	120	+
C_8	Caplylic	1/1000	20	+	2.5	±	5	+	1	−
	Suberin.	1/4000	4320	±	1440	+	2160	+	90	+
C_9	Pelargonic	1/1000	20	±	2.5	±	5	+	1	−
	Azelainic.	1/4000	2160	+	720	+	1440	+	90	±
C_{10}	Capric	1/1000	20	±	2.5	±	5	+	1	−
	Sebacinic	1/4000	720	±	350	±	720	±	45	±

In table V, line of concentration shows the concentration of each acids due to pH 3.0, pH 4.0 or pH 4.8.

From the results noted in Table V (1) and (2), we find the following. If we compare the sterilizing action of acids of C_2 to C_{10} at the same pH, we find that saturated monobasic fatty acids have the strongest sterilizing action, unsaturated fatty acids have the next and dibasic fatty acids have the weakest sterilizing action.

The fact is distinctly according to the increase of C atom. If we compare the molecular concentration of each acids, concentration of dibasic fatty acids is smaller than those of monobasic fatty acids.

By this fact we find that difference of sterilizing action of the same pH solution of fatty acids, due to the difference of molecular concentration of each acids.

Summary.

I studied the sterilizing action of dibasic fatty acids $C_nH_{2n}\cdot(Co_2H)_2$ on putrifactive bacteria, Bac. typhosus and Vib. cholerae, and also compared the sterilizing action between dibasic and monobasic fatty acids.

Results are as follows:

(1) At the same molecular concentration, oxalic acid (C_2) is the strongest, malonic acid (C_3) is the next, and from malonic acid the sterilizing power suddenly greatly diminishes. From C_6 acid the sterilizing action of acids gradually increases, according to the increase of the number of C atom.

(2) Anions of C_2 to C_6 acids have no sterilizing action. According to the increase of the number of C atom from C_6 acid the sterilizing power of anions gradually increases, but its action is very weak.

(3) Among salts of Na, Ca and NH_4, Ca salts have a somewhat weak

sterilizing power, and NH_4 salts have a somewhat long surviving period for bacteria.

(4) At the same molecular concentration of acids having the same number of C atom, from C_2 to C_3 acids, dibasic fatty acid is the strongest, unsaturated monobasic fatty acid is the next, and saturated monobasic fatty acid is the weakest. The fact is chiefly due to pH. From C_6 acid, according to the increase of the number of C atom, the sterilizing action of monobasic fatty acids are stronger than those of dibasic fatty acids. The fact due to the effect of undissociated molecules of each acids.

(5) At the same pH, sterilizing action of saturated monobasic fatty acid is the strongest, unsaturated monobasic fatty acid is the next and didasic fatty acid is the weakest of all. The fact is due to the difference of molecular concentration of each acids.

(6) There is no special relation between the number of CO_2H group and the strength of sterilizing action.

Refference.

(1) S. TETSUMOTO: The Bul. of the Agr. chem. S. of Iap. Vol. 9, Nos. 1~3, (1933).
(2) 〃 〃 〃 〃 Nos. 3~5, (1933).

Bombilupeol, a Colorless Crystallizable Constituent of the Alcoholic Extract of the Domestic Cocoon, (Résumé)

By

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In the course of my investigation regarding the natural coloring matters of the yellowish green domestic cocoon named "Seihaku", a colorless substance which gave the color reactions of phytosterol was isolated from the ether soluble fraction of the cold alcoholic extract of the cocoon layer in the pure crystallized form and found to be identical with lupeol by its molecular formula and other properties.

The auther decided to give the name of "Bombilupeol" to the substance because no one has yet isolated it not only from domestic cocoon (Bombyx mori), but also from animal origin.

Experimental.

The cold 70% ethyl alcoholic extract of the cocoon layer was condensed and fractionated into two parts, namely, the ether soluble in which bombilupeol dissolved and the aqueous in which a great part of the yellowish green pigment was dissolved. From the none-saponifiable fraction of the ether soluble part, the pure substance was isolated after repeating the recrystallization from methanol, using animal charcoal. The yield of the pure crystal was about 0.01% of the cocoon layers.

The substance crystallized out as colorless, needle shaped crystals, sometimes forming radiant colonies with silky appearance. When crystallized out from ether, it formed needles arranged in sheaves. The pure crystal melted sharply at 213° (corr.) and showed no depression of the melting point by mixing in it the lupeol which was isolated from the bark of "Sikeihi". From the results of analysis the molecular formula was given as $C_{30}H_{50}O$ with one double bond. The rotation in chloroform solution was estimated as $[\alpha]_D^{25} = +27.50°$. The substance was insoluble in water, acids or alkalies, sparingly soluble in cold alcohol but soluble in hot alcohol and readily soluble in cold ether, chloroform, benzene and petroleum ether and gave, after long elapse of time, the color reactions of Liebermann-Burchard and others, which being characteristic to phytosterol. On treatment with acetic acid anhydride and benzoyl chloride, the substance was converted respectively into acetate (M. P. 216°) and benzoate (M. P. 270°).

The trial to detect the bombilupeol in the other species of domestic cocoon, eg., yellowish, pale green and white, gave negative results, but a minute quantities of substances gave color reactions of phytosterol was isolated, leaving its further examination in future.

The origin of bombilupeol of the yellowish green cocoon can be presumed as deriving from the mulberry leaves on which silk warm feeds but its detection in the leaves must be carried out by further experiment.

Über die Bildung des Harnstoffs aus Uraminosäuren, Hydantoinen und aus Eiweisskörpern durch Einwirkung von Enzymen (Reduktasen) in neutraler Lösung.

Von

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(Eingengangen am 17, Januar 1934)

In der vorigen Arbeit(1) hat der Verfasser mitgeteilt, dass der Harnstoff aus Citrullin, Prolysin, verschiedenen Hydantoinen, sowie aus Eiweisskörpern durch Einwirkung von Schwefelwasserstoff in schwach alkalischer Lösung gebildet wird.

In weiterer Fortsetzung dieser Arbeit hat der Verfasser beobachtet, dass in Milch, Blut, Pankreas oder Leber ein gewisses Enzym vorhanden ist, welches die Fähigkeit besitzt aus den obengenannten Substanzen Harnstoff abzuspalten. Es sei nur erwahnt dass in frischer Milch kleine Menge Harnstoff ursprünglich vorhanden ist. So wurde in einem Versuche, aus 1850 ccm frischer Milch 0.85 g roher Harnstoff isoliert. Durch Sterilisieren bei 100° nimmt er aber nicht zu. In Blut, Pankreas oder Leber lasst sich auch etwas Harnstoff nachweisen.

Wenn man eine bestimmte Menge Citrullin, Prolysin oder Hydantoinen in frischer Milch zusetzt und 1~2 Stunden bei 60~70° stehen lasst, so beobachtet man immer die Vermehrung des Harnstoffs. Wenn aber anstatt frischer Milch bei 100° sterilisierte Milch verwendet wird, so findet keine Vermehrung des Harnstoffs statt. Daraus kann man schliessen dass das Enzym in Milch, aus Citrullin, Prolysin usw. Harnstoff abgespalten hat.

Dieselbe Versuche wurden auch mit denselben Resultate mit Blut, Pankreas und Leber wiederholt.

Obgleich die Natur des betreffenden Enzyms noch nicht klar ist, nehmen wir doch an, dass es sich um eine Reduktase handelt, weil die Bildung des Harnstoffs mit der reduzierenden Wirkung der Milch, Blut usw. beinahe parallel geht.

Experimenteller Teil.

(1) 1850 ccm frische Milch wurden im Vakuum bis zum Trocknen eingedampft, und mit Methylalkohol extrahiert. Die Methylalkoholishe Lösung wurde wieder eingedampft und nach Behandlung mit Petroleumäther, mit

Äther wiederholt ausgezogen. Beim Verdampfen des Äthers schied sich der Harnstoff kristallinisch aus. Der letztere war in Wasser und Alkohol löslich und gab die P. Ehrlichsche sowie die Schiffsche Reaktion sehr stark. Durch Einwirkung von Urease in wässeriger Lösung wurde er in Ammoniak gespalten. Zur weiteren Identifizierung wurde er in Nitrat, Oxalat und in Dixanthyl-derivat verwandelt. Die Ausbeute an rohem Harnstoff betrug 0,85 g.

(2) 0,0512 g Citrullin wurden in 25 ccm frischer Milch gelöst, mit soviel *n*/10 Kalilauge versetzt bis die Lösung gegen Phenolrat neutral reagierte und eine Stunde auf dem Wasserbade bei 65° erwärmt. Nach dem Erkalten wurde der gebildete Harnstoff in Ammoniak verwandelt, indem die Lösung mit 1 ccm 10%iger Urease Lösung versetzt, mit wenig flüssigem Paraffin überschichtet und bei Zimmertemperatur stehen gelassen wurde. Nach 30 Minuten wurden das dadurch gebildete Ammoniak in bekannter Weise mit *n*/10 Schwefelsäure titriert.

In dieser Weise wurde gefunden:

	n/10 H_2SO_4 verbraucht zur Titration (ccm)	Berechnet für Harnstoff (mg)	% der Theorie
1) 25 ccm Milch + 0,0512 g Citrullin	4,2	11,735	
2) 25 ccm Milch ohne Zusatz von Citrullin	2,7	7,544	
3) NH_3 gebildet aus Citrullin (1−2)	1,5	4,191	23,86

Im folgenden werden die Resultate der in analoger Weise mit verschiedenen Substanzen ausgeführten Versuche tabellarisch zusammengestellt:

	n/10 H_2SO_4 verbraucht zur Titration (ccm)	Berechnet für Harnstoff (mg)	% der Theorie
1) 25 ccm frischer Milch	2,7	7,544	
2) " sterilizierte Milch	2,7	7,544	
3) " frischer Milch nach 1 Tag	3,0	8,382	
4) " " " " 1 Woche	3,7	10,337	
5) " sterilizierte Milch + 0,1024 g Carbamylalanin	2,7(2,7+0,0)	0,0	0,0
6) " " " + 0,1116 g Phenylalaninhydantoin	2,7(2,7+0,0)	0,0	0,0
7) " frischer Milch + 0,0512 g Citrullin	4,2(2,7+1,5)	4,191	23,86
8) " " " + 0,1010 g Carbamylleucin	4,5(2,7+1,8)	5,029	14,44
9) " " " + 0,1003 g Carbamylphenylalanin	5,2(2,7+2,5)	6,985	24,13
10) 50 ccm Milch (nach 1 Woche) + 0,0328 g Carbamylphenylalanin (erwärmt 2 St. 30 Min.)	7,8(3,7×2+0,4)	1,118	11,81
11) 25 ccm Milch + 0,0996 g Prolysin	3,8(2,7+1,1)	3,073	11,05

12)	25 ccm Milch + 0,10036 g Leucinhydantoin	3,4(2,7 + 0,7)	1,956	5,07
13)	″ ″ + 0,10022 g Phenylalaninhydantoin	3,3(2,7 + 0,6)	1,676	5,30
14)	″ ″ + 4,0 ccm Ovalbumin	3,4(2,7 + 0,7	1,956	
15)	″ ″ + 3,0 g Gelatine	3,8(2,7 + 1,1)	3,074	

1 ccm H_2SO_4 = 4,561 mg H_2SO_4 = 2.794 mg Harnstoff

		H_2SO_4 (ccm) verbraucht zur Titration	Berechnet für Harnstoff (mg)	% der Theorie
1)	25 ccm Blutserum des Pferdes	4,6	12852	
2)	″ ″ (erhitzt 30 Minuten bei 100°)	4,6	12852	
3)	″ ″ (″ ″ ″ ″ ″) + 0,1154 g Carbamylphenylalanin	4,6 (4,6 + 0,0)	0,0	0,0
4)	25 ccm Blutserum + 0,1002 g Carbamylleucin	5,1 (4,6 + 0.5)	1,397	4,04
5)	″ ″ + 0,1016 g Carbamylphenylalanin	5,5 (4,6 + 0,9)	2,515	8,58
6)	″ ″ + 0,0996 g Prolysin	6 5 (4,6 + 1,9)	5,308	19,09
7)	″ ″ + 0,1024 g Leucinhydantoin	6,3 (4,6 + 1,7)	4,749	12,06
8)	″ ″ + 0,1006 g Phenylalaninhydantoin	6,2 (4,6 + 1,6)	4,470	14,07
9)	″ ″ + 2,0 ccm Ovalbumin	5,0 (4,6 + 0,4)	1,118	
10)	″ ″ + 1,5 g Gelatine	5,0 (4,6 + 0,4)	1,118	
1)	10 ccm Pankreassaft*	1,5	4,191	
2)	″ ″ (erhitzt 30 Minuten bei 100°)	1,5	4,191	
3)	″ ″ (″ ″ ″ ″ ″) + 0,1075 g Carbamylphenylalanin	1,5 (1,5 + 0,0)	0,0	0,0
4)	10 ccm Pankreassaft + 0,0996 g Prolysin	2.4 (1,5 + 0,9)	2,515	9,04
5)	″ ″ + 0,10036 g Leucinhydantoin	2,3 (1,5 + 0,8)	2,235	5,80
6)	″ ″ + 0,10022 g Phenylalaninhydantoin	2,4 (1.5 + 0,9)	2,515	7,94
7)	10 ccm Pankreassaft + 4,0 ccm Ovalbumin	1,8 (1,5 + 0,3)	0,838	
8)	″ ″ + 3,0 g Gelatine	1,9 (1,5 + 0,4)	1,118	
1)	10 ccm Lebersaft*	1,2	3,352	
2)	″ ″ (erhitzt 30 Minuten bei 100°)	1,2	3,352	
3)	″ ″ (″ ″ ″ ″ ″) + 0,1035 g Carbamylphenylalanin	1,2 (1,2 + 0,0)	0,0	0,0
4)	10 ccm Lebersaft + 0,0996 g Prolysin	2,0 (1,2 + 0,8)	2,235	8,04
5)	″ ″ + 0,10036 g Leucinydantoin	1,9 (1,2 + 0,7)	1,955	5,07
6)	″ ″ + 0,10022 g Phenylalaninhydantoin	1,9 (1,2 + 0,7)	1,955	6,18
7)	10 ccm Lebersaft + 4,0 ccm Ovalbumin	1,5 (1,2 + 0,3)	0,838	
8)	″ ″ + 3,0 g Gelatine	1,6 (1,2 + 0,4)	1,118	

* 500 g Subst. extrahiert mit 100 ccm H_2O bei 70°.

Man sieht dass der Harnstoff aus verschiedenen Substanzen gebildet worden ist. Die Reaktion verlief aber nicht quantitativ, so dass die erhaltenen Werte viel niedriger waren als man erwartete.

Im nachsten Versuche wurde dem Reaktionsgemisch etwas Formaldehyd zugesetzt und zwar in folgender Weise:

2000 ccm Milch wurden mit 16 ccm gesattigter Lösung der Citronensäure versetzt, der entstandene Niederschlag abfiltriert. 100 ccm des Filtrats wurden mit 0,1000 g Carbamylphenylalanin versetzt und mit $n/10$ Kalilauge bis zu schwachsauer Reaktion neutralisiert und nach dem Zusatz von 1 ccm kauflichem Formaldehyd bei 35° stehen gelassen. Nach einem Tage wurde der gebildete Harnstoff in obenerwahnter Weise in Ammoniak verwandelt und mit $n/10$ Schwefelsaure titriert.

Die Resultate sind wie folgendes:

	$n/10$ H_2SO_4 verbraucht zur Titration (ccm)	Berechnet für Harnstoff (mg)	% der Theorie
1) 100 ccm des Filtrats + 0,1000 g Carbamylphenylalanin	27,5	76,835	
2) 100 ccm des Filtrats ohne Zusatz von Carbamylphenylalanin	21,1	58,953	
3) NH_3 gebildet aus Carbamylphenylalanin (1−2)	6,4	17,882	62,00

Ferner wurde das obenerwahnte Filtrat im Vakumn bis zum Ein fünftel Teil eingedampft und 50 ccm davon wurden mit carbamylphenylalanin versetzt und nach dem Zusatz von 1 ccm Formaldehyd 24 Stunden bei 35° stehen gelassen. In diesem Fall erhielt man etwas bessere Resultate wie aus folgender Tabelle zu ersehen ist.

	$n/10$ H_2SO_4 verbraucht zur Titration (ccm)	Berechnet für Harnstoff (mg)	% der Theorie
1) 50 ccm des obenerwähnten Konzentrats + 0,1026 g Carbamylphenylalanin	26,2	73,202	
2) 〃 〃 〃 〃 〃 + 0,1002 g Carbamylleucin	27,6	77,114	
3) 〃 〃 〃 〃 〃 allein ohne Zusatz	17,5	48,895	
4) NH_3 gebildet aus Carbamylphenylalanin (1−3)	8,7	24,307	8[illegible]5
5) 〃 〃 〃 Carbamylleucin (2−3)	10,1	28,219	81,68

Literature.

(1) Proc. Imp. Acad. 9, 305~308, (1933).

Untersuchungen über die Enzyme von Bombyx mori L. IV. Mitteilung.

Über die Phenolasen des Blutes.

Von

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Über die Fermente bei Wirbellosen sind veraltete zahlreiche Angaben vorhanden; moderne, quantitative Arbeiten aber sehr sparlich. Als Versuchstier ein Zuchtinsekt, die Seidenraupe, benutzend, hat der Verf. bereits einige Studien über mehrere Fermente des Verdauungssaftes und Blutes derselben angestellt(4).

Die physiologische Bedeutung der Desmolasen sowie die gegenseitigen Beziehungen zwischen ihren Teilfermenten sind heute noch nicht ganz klar. Es wurde in meiner vorhergehenden Mitteilung(5) gezeigt, dass die Tyrosinase und Katalase des Blutes von Bombyx mori besonders im Laufe von drei Entwicklungsperioden sehr auffallend sich verandern. Diese bemerkenswerten Tatsachen haben mich weiter zu Untersuchungen der anderen Oxydationsfermente, der Phenolasen, des Blutes des gleichen Insekts veranlasst.

I. Eigentliche oxydatische Wirkung.

1) Methodik:- Ich wahlte die Rasse Japan-110-G für meine Versuche aus, die im Sommer 1933 aufgezogen wurde. Die Messung der Fermentmenge geschah durch die Oxydation von Guajacol. Zu diesem Zweck wurden 2 ccm 1 proz Guajacollösung, 1 ccm M/3 Phosphatpufferlössung von Ph 6.6, 6 ccm Wasser und 1 ccm 20 fach verdünntes Blut gemischt und 30 minuten lang bei 30° gehalten. Dann wurde die Versuchslösung bei 0° abgekühlt und sofort die entstandene Färbung kolorimetrisch bestimmt. Die in diesem Falle nötige Standardsfarbenskala kann nach der Beschreibung von Bansi und Ucko(2) hergestellt werden.

2) *Optimale Ph.*

Ph	Oxyd. G. mg	Oxydation, %	Ph	Oxyd. G. mg	Oxydation, %
5.3	5.83	29.15	6.8	6.48	32.40
5.9	6.25	31.25	7.0	6.48	32.40
6.2	6.73	33.65	7.4	6.25	31.25
6.6	7.00	35.00	8.0	6.03	30.15

G = Guajacol.

3) *Optimaltemperatur.*

Temp.	Oxyd. G. mg	Oxydation, %	Temp.	Oxyd. G. mg	Oxydation, %
0	0.35	1.75	30	1.25	6.25
10	0.87	4.35	40	1.51	7.55
20	1.04	5.02	50	1.27	6.35

4) *Änderungen durch Hungerzustand bei den Raupen.*

Hungerstunden	1	6	25	52
Oxyd. G. mg	0.45	1.27	0.23	0.13
Oxydation, %	2.25	6.35	1.15	0.65

5) *Unterschied zwischen gesunden und kranken Raupen.*

	Gesunde Raupen			Krank. Raup. (Nankabyo)		
	Gruppe, 1	Gruppe, 2	Mittel	Gruppe, 1	Gruppe, 2	Mittel
Oxyd. G. mg	0.21	0.18	0.20	0.47	0.42	0.45
Oxyd. %	1.05	0.90	1.00	2.35	2.10	2.25

6) *Veränderungen im Laufe von drei Entwicklungsperioden von Bombyx mori.*

		♀		♂	
	Tage	Oxyd. G. mg	Oxyd. %	Oxyd. G. mg	Oxyd. %
Raupe	V. Lebensalter 2	5.28	26.40	5.51	27.55
	4	0.45	2.25	2.39	11.95
	7	0.53	2.65	0.21	1.05
Einspinnen des Kokons	2	0.57	2.85	0.23	1.15
	4	0.44	2.20	0.20	1.00
Puppe	1	0.41	2.05	0.35	1.75
	3	0.82	4.10	0.37	1.85
	5	0.92	4.60	0.53	2.65
	7	1.21	6.05	0.37	1.85
	10	0.33	1.65	0.24	1.20
Schmetterling	1	15.40	77.06	16.22	81.10
	2	16.22	81.10	——	——

II. Peroxydatische Wirkung.

1) *Methodik*:– Die Untersuchungen wurden nach der Methode von Bach und Zubkowa⁽¹⁾ ausgeführt, und zwar mit einige Abänderungen. 2 ccm 1 proz. Guajacollösung, 1 ccm M/3 Phosphatpuffermischung von Ph 6.6, 1 ccm

0.2 proz. H_2O_2-Lösung, 5 ccm Wasser und 1 ccm 20fach verdünntes Blut wurden vermischt. Nach 30 Minuten langer Erwärmung auf 30° wurde die Versuchslösung auf 0° abgekühlt und die Abstufung der entstandenen Farbe kolorimetrisch bestimmt.

2) *Optimale Ph.*

Ph	Oxyd. G. mg	Oxyd. %	Ph	Oxyd. G. mg	Oxyd. %
5.3	5.83	29.15	6.8	5.93	29.65
5.9	6.03	30.15	7.0	5.93	29.65
6.2	6.03	30.15	7.4	5.85	29.15
6.6	6.14	30.70	8.0	5.64	28.20

3) *Optimaltemperatur.*

Temp.	Oxyd. G. mg	Oxyd. %	Temp.	Oxyd. G. mg	Oxyd. %
0	0.52	2.62	30	0.71	3.55
10	0.69	3.45	40	0.54	2.70
20	0.94	4.70	50	0.35	1.75

4) *Änderungen durch Hungerzustand bei den Raupen.*

Hungerstunden	2	7	24	51
Oxyd. G. mg	0.50	0.71	0.22	0.11
Oxydation, %	2.50	3.55	1.10	0.55

5) *Unterschiedzwischen gesunden und kranken Raupen.*

	Gesunde Raupen			Krank. Raup. (Nankabyo)		
	Gruppe, 1	Gruppe, 2	Mittel	Gruppe, 1	Gruppe, 2	Mittel
Oxyd. G. mg	0.15	0.13	0.14	0.41	0.35	0.38
Oxyd. %	0.75	0.65	0.70	2.05	1.75	1.90

6) *Veränderungen im Laufe von drei Entwicklungsperioden von Bombyx mori.*

		♀		♂	
	Tage	Oxyd. G. mg	Oxyd. %	Oxyd. G. mg	Oxyd. %
Raupe	V Lebensalter 2	3.67	18.35	4.40	22.00
	4	0.50	2.50	0.54	2.70
	7	0.32	1.60	0.19	0.95
Einspinnen des Kokons	2	0.39	1.95	0.23	1.15
	4	0.41	2.05	0.26	1.30

Puppe	1	0.37	1.85	0.34	1.70
	3	0.73	3.65	0.35	1.75
	5	0.88	4.40	0.50	2.50
	7	1.16	5.80	0.35	1.75
	10	0.29	1.49	0.24	1.20
Schmetterling	1	16.22	81.10	19.35	96.75
	2	17.94	89.70	——	——

III. Diskussion.

Aus allen oben angeführten und in der dritten Mitteilung beschriebenen Versuchen ergibt es sich, dass verschiedene physiologische bezw. pathologische Verhaltnisse bei Bombyx mori in der Regel erhebliche Schwankungen des Gehaltes an Oxydationsfermenten im Blute bedingen. Vor allem können sehr bedeutende, verhaltnismassig regelmassige Änderungen der Blutfermente bei Metamorphose als ein geeignetes Material für die Besprechung über die noch wenig bekannten, gegenseitigen Beziehungen zwischen den verschiedenen Oxydationsfermenten dienen.

Welcher Unterschied besteht zwischen den sogenannten wahren Oxydasen und Peroxydasen? Oppenheimer(5) fasste diese beiden enzymatischen Wirkungen auf die Polyphenole als Phenolasen zusammen. Verf. prüfte bei Bombyx mori die oxydatische und peroxydatische Wirkung des Blutes auf Guajacol und beobachtete, dass diese beiden Wirkungen in manchen Fallen gleichlaufend sich verandern.

Die Grenze zwischen der Wirkung der Phenolasen und der der Tyrosinase ist noch nicht entscheidend. Es wurde auch gefunden, dass Hunger und besonders Metamorphose bei Bombyx mori im allgemeinen parallele Steigerungen oder Senkungen dieser beiden Fermente des Blutes hervorbringen.

Ferner ist die Frage, ob in den Lebewesen ein Parallelismus zwischen dem Vorkommen der Peroxydase und der Katalase existiert oder nicht, vielfach erörtert worden. Bei Bombyx mori konnte ich solchen Parallelismus nicht beobachten. Es ist bemerkenswert, dass die Blutkatalase mit dem Beginn des Kokonspinnens der Larve bedeutend starker wird, wahrend die Blutperoxydase bei den Puppen sehr schwach ist.

(1) Die Phenolasen des Blutes von Bombyx mori wirken am besten bei Ph 6.6. Die Optimaltemperatur der eigentlichen oxydatischen Wirkung liegt bei 40° und diejenige der peroxydatischen bei 20°.

(2) Durch Hunger bei Raupen werden die Phenolasen erst etwas stärker und dann schwächer. Bei kranken Raupen (Nankabyosan) sind diese enzy-

matischen Wirkungen recht viel höher als bei gesunden.

(3) Die Wirkungen der Phenolasen sind im ersten Stadium des fünften Lebensalters der Larve beträchtlich aktiv, sinken dann aber allmählich. Während der Puppenperioden bleiben die Phenolasen bedeutend zurück und in den Tagen des Schmetterlingslebens vermehren sie sich rasch bis zu einem Maximum.

(4) Der Phenolasengehalt des Blutes im Larven- und Schmetterlingsstadium ist bei den Männchen etwas höher als bei den Weibchen, im Puppenstadium dagegen bei den Weibchen etwas höher als bei den Männchen.

(5) Es wurde in meinen Untersuchungen bestätigt, dass die Oxydationsfermente des Blutes im Laufe von drei Entwicklungsperioden von Bombyx mori sehr auffallend sich verändern, und zwar die Tyrosinase, eigentliche Oxydase und Peroxydase in der Regel miteinander gleichlaufend, aber die Katalase allein gegen dieselben vielmehr umgekehrt.

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(3) C. Oppenheimer: Die Fermente u. ihre Wirkungen, 5. Aufl.
(4) K. Yamafuji: Bull. Agr. Chem. Soc. Japan, **8**, 156, (1932); **9**, 19, (1933); **9**, 172, (1933).
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Untersuchungen über die Enzyme von Bombyx mori L. V. Mitteilung.

Über die Saccharase und Maltase des Blutes.

Von

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(Eingegangen am 5, Januar 1934)

In früheren Mitteilungen⁽¹⁾ hat der Verf. die merkwürdigen Beziehungen der verschiedenen physiologischen bezw. pathologischen Verhältnisse bei Bombyx mori zu den Schwankungen des Gehaltes an Oxdationsfermenten im Blute beschrieben. Da kein quantitativer Versuch über die Disaccharasen des Blutes der Seidenraupe existiert, wurde nun von demselben Autor ein Studium auf diesem Gebiet vorgenommen.

I. Über die Saccharase.

1) *Methodik*:– Zu dem nachstehend erwähnten Experimenten wurden drei Originalrassen der Seidenraupen, namlich Japan-110-E, China-7-B und Europa-7-C, gebraucht. Die von mir angewandte Methodik für die quantitative Bestimmung der Fermentaktivität ist die folgende:

Das Totalvolumen der Proben betrug stets 13 ccm, wovon 10 ccm 10 proz. Saccharoselösung, 2 ccm M/3 Phosphatpufferlösung von Ph 6.8 und 1 ccm zweifach verdünntes Blut. Diese Reaktionsmischung, 24 Stunden bei 37° erwärmt, wurde mit 3 ccm 2 *N* Sodalösung versetzt und dann das Drehungsvermögen der erhaltenen Lösung polarimetrisch ermittelt.

2) *Optimale Ph.*

Ph	Drehungsabnahme, °	Saccharosespaltung, %	Ph	Drehungsabnahme, °	Saccharosespaltung, %
5.3	0.92	17.62	6.8	1.02	19.54
5.9	0.95	18.20	7.0	0.99	18 97
6.2	1.10	21.07	7.4	0.93	17.82
6.6	1.14	21.84	8 0	0 91	17.43

3) *Unterschied zwischen gut gewachsenen und schlecht gewachsenen Raupen.*

	Gut gewachs. Raupen		Schlecht gewachs. Raup.	
	♀	♂	♀	♂
Dreh.-abnahme, °	2.82	2.59	3.10	2.12
Sacch.-spalt. %	54.02	49.62	59.39	52 11

4) *Änderungen durch Hungerzustand bei den Raupen.*

	♀		♂	
Hungerstunden	Dreh.-abn. °	Sac.-sp. %	Dreh.-abn. °	Sac.-sp. %
2	1.69	31.23	1.16	22.22
24	1.47	28.16	1 15	22.03
48	1.22	23.37	0.96	18.37

5) *Unterschied zwischen gesunden und kranken Raupen.*

	Gesunde Raupen		Krank. Raup. (Nankabyo)	
	♀	♂	♀	♂
Dreh.-abnahme, °	1.70	1.48	0.97	1.27
Sacch.-spalt. %	32.57	28.35	18.58	24.33

6) Veränderungen im Laufe von drei Entwicklungsperioden von Bombyx mori.

	Tage	♀		♂	
		Dreh.-abn. °	Sac.-sp. %	Dreh.-abn. °	Sac.-sp. %
Raupe	V Lebensalter 3	3.87	74.14	3.81	72.99
	5	3.47	66.48	3.40	65.13
	8	2.91	55.75	2.65	50.77
Einspinnen des Kokons	2	2.35	45.02	2.38	45.59
	4	2.41	41.00	2.60	49.81
Puppe	2	1.69	32.38	1.57	30.08
	7	1.32	25.29	1.21	23.18
	9	1.21	13.18	1.08	20.69
Schmetterling	1	1.08	20.69	—	—

7) Unterschied zwischen drei Originalrassen der Seidenraupen.

V Lebensalter, Tage	Japan 110-E		China-7-B		Europa-7-C	
	♀ Sac.-sp. %	♂ Sac.-sp. %	♀ Sac.-sp. %	♂ Sac.-sp. %	♀ Sac.-sp. %	♂ Sac.-sp. %
3	74.14	72.99	19.54	17.62	43.49	41.59
5	66.48	65.13	31.23	22.22	35.63	36.40
Reife	55.75	50.77	32.57	28.35	35.82	42.15

II. Über die Maltase.

1) Methodik:– Die Versuche erfolgten mittels folgendem Verfahren. Zur Mischung der 10 ccm 5 proz. Maltoselösung und der 2 ccm M/3 Phosphatpufferlösung von Ph 6.8 wurde 1 ccm Blut hinzugefügt und während 24 Stunden bei 37° stehen gelassen. Dann wurde die Reaktion durch 3 ccm 2 *N* Sodalösung sistiert und der Drehungsgrad der erhaltenen Lösung beobachtet.

2) Optimale Ph.

Ph	Drehungsabnahme, °	Maltosespaltung, %
5.3	0.08	3.51
5.9	0.06	2.63
6.2	0.05	2.19
6.6	0.15	6.40
6.8	0.13	5.70
7.0	0.12	5.26
7.4	0.10	4.39
8.0	0.07	3.07

3) *Unterschied zwischen gut gewachsenen und schlecht gewachsenen Raupen.*

	Gut gewachsene Raupen		Schlecht gew. Raupen	
	♀	♂	♀	♂
Dreh.-abnahme, °	0.22	0.18	0.23	0.20
Malt.-spalt. %	9.65	7.89	10.09	8.77

4) *Änderungen durch Hungerzustand bei den Raupen.*

	♀		%	
Hungerstunden	Dreh -abn. °	Malt.-sp. %	Dreh.-abn. °	Malt.-sp. %
2	0.23	10.09	0.21	9.21
24	0.17	7.46	0.19	8.33
48	0.20	8.77	0.22	9.65

5) *Unterschied zwischen gesunden und kranken Raupen.*

	Gesunde Raupen		Krank. Raup. (Nankabyo)	
	♀	♂	♀	♂
Dreh.-abnahme, °	0.27	0.22	0.28	0.24
Malt.-spalt. %	11.84	9.65	12.28	10.53

6) *Veränderungen im Laufe von drei Entwicklungsperioden von Bombyx mori.*

	Tage		♀		♂	
	Tage		Dreh.-abn. °	Mal.-sp. %	Dreh.-abn. °	Mal.-sp. %
Raupe	V. Lebensalter	3	0.09	3.95	0.07	3.07
		5	0.07	3.07	0.06	2.63
		8	0.23	10.09	0.19	8.33
Einspinnen von Kokons	2		0.26	11.40	0.28	12.28
	4		0.38	16.67	0 24	10.53
Puppe	2		0.20	8.77	0.18	7.89
	7		0.14	6.14	0.10	4.39
	9		0.10	4.39	0.08	3.51
Schmetterling	1		0.36	15.79	——	——

7) *Unterschied zwischen drei Originalrassen der Seidenraupen.*

	Japan-110-E		China-7-B		Europa-7-C	
V. Lebensalter, Tage	♀ Mal.-sp. %	♂ Mal.-sp. %	♀ Mal.-sp. %	♂ Mal.-sp. %	♀ Mal.-sp. %	♂ Mal.-sp. %
3	3.95	3.07	9.21	7.46	5.70	3.95
5	3.07	2.63	10.09	9.21	7.46	3.07
Reife	10.09	8 33	8.77	9.65	14.35	9.65

III. Diskussion.

Bei höheren Tieren scheinen im allgemeinen die Blutfermente in der normalen Assimilation der Nahrungsmittel und bei dem Abbau der resorbierten Stoffe einehöchst geringe Rolle zu spielen. Bei niederen Tieren, wie Bombyx mori, müssen aber die Verhaltnisse etwas andere sein.

Die in vorangegangenen Untersuchungen(2) über die Verdauungs- und Blutfermente gewonnenen Ergebnisse drangen zu der Annahme, dass die Hydrolasen des Blutes bei der Seidenraupe an der Verdauung bezw. am Stoffwechsel ein wichtigen Anteil nehmen.

Die in der vorliegenden Arbeit mitgeteilten Versuche dürften die Richtigkeit dieser Voraussetzung bestatigen.

Trotzdem das Maulbeerblatt eine ziemliche Menge Rohrzucker enthalt, ist im Digestionssafte der Seidenraupe keine Saccharase vorhanden. Eine sehr schwache Saccharase findet sich in der Darmwand, aber eine recht viel starkere im Blut derselben. Durch Hunger vermindert sich bei der Larve die Blutsaccharase. Dasselbe Ferment wird vom Tage des Einspinnens in den Kokon bis zu den Tagen des Schmetterlings allmahlich verringert.

Auf Grund dieser Tatsachen dürfte der Schluss gerechtfertigt sein, dass die Blutsaccharase bei Bombyx mori an der Aufspaltung des Rohrzuckers der Nahrung beteiligt ist.

Bekanntlich ist in frischem Maulbeerblatt Starkemehl in bedeutender Menge enthalten, wahrend der Malzzucker durch die Fermente des Digestionskanals der Seidenraupe kaum gespalten werden kann. Im Blute derselben kommt indessen eine schwache Maltase vor. Eine gewaltige Vermehrung der Glykogenvorräte findet im fünften Lebensalter der Larve statt, aber eine allmählige Verminderung derselben in den Puppen- und Schmetterlingsperioden von Bombyx mori. Die Wirkung der Blutmaltase ist in den Tagen der Kokonspinnens der Raupe am starksten, wird im Laufe der Puppenperiode von Tag zu Tag schwacher und dann in der Periode des Schmetterlingslebens wieder gesteigert. Innerhalb der zwei Hungertage bei der Larve verändert sich die Blutmaltase nicht wesentlich.

Es geht also daraus hervor, dass die Blutmaltase bei Bombyx mori an der Malzzuckers aus der Nahrungsstärke sowie aus dem Körperglykogen Anteil nimmt.

Zusammenfassung.

(1) Im Blute von Bombyx mori kommt eine wirksame Saccharase vor, deren Wirkungsoptimum bei Ph 6.6 liegt.

(2) Die Saccharase des Blutes ist bei gesunden Raupen recht viel stärker als bei kranken (Nankabyosan) und auch bei schlecht gewachsenen Raupen etwas höher als bei gut gewachsenen. Durch Hunger ist sie vermindert.

(3) Die Blutsaccharase ist im fünften Lebensalter sehr aktiv, aber dann sinkt sie in den Puppen- und Schmetterlingsperioden allmählich. Die Saccharase des Weibchens ist immer stärker als die des Männchens. Es besteht die folgende Reihenfolge für die Wirksamkeit der Blutsaccharase: Japan-110-E > Europa-7-C > China-7-B.

(4) Die Blutmaltase von Bombyx mori wirkt am besten bei Ph 6.6.

(5) Die Maltasewirkung ist bei kranken Raupen (Nankabyosan) etwas höher als bei gesunden und auch bei schlecht gewachsenen Raupen ein wenig stärker als gut gewachsenen. Durch Hunger verändert sie sich nicht wesentlich.

(6) Im Laufe der drei Entwicklungsperioden ist die Blutmaltase in den Kokonspinnens am aktivesten, wird während der Puppenperiode allmählich schwächer und ist in den Tagen des Schmetterlingslebens wieder bedeutend stärker. Die Maltase des Weibchens ist stets aktiver als die des Mannchens. Es besteht kein wesentlicher Unterschied in der Maltasewirksamkeit der drei Rassen.

(7) Aus den oben erwähnten und anderen Tatsachen lässt sich schliessen, dass diese beiden Blutdisaccharasen bei Bombyx mori für die Verdauung bezw. den Stoffwechsel eine wichtige Rolle spielen.

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Synthesis of 5-Oxy-camphor.

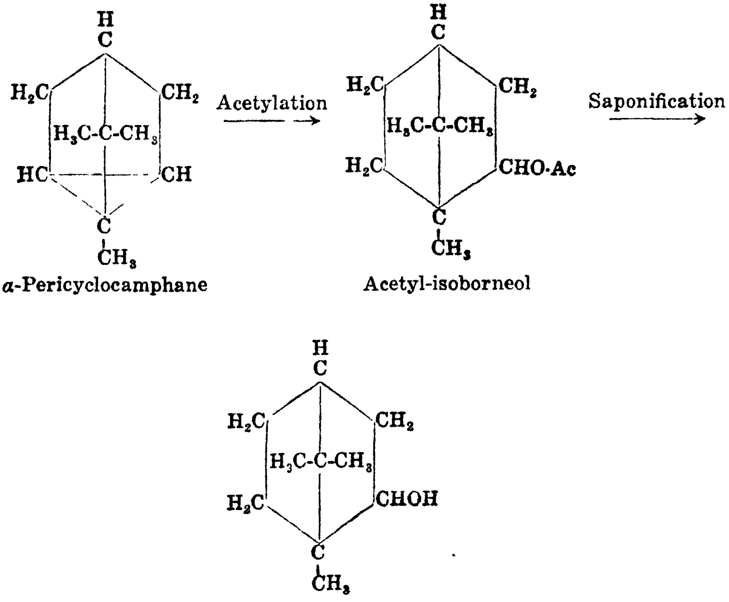
By

Kunijiro TAKEUCHI.

(Received January 8, 1934)

According to Y. Asahina and M. Ishidate(1) 5-oxy-camphor is a chief component of "Campherol"(2) which is obtained from the urine of dogs administered with camphor. The present author has tried to prepare it synthetically.

L. Ruzicka and Liebi obtained isoborneol(3) according to Bertram and Walbaum's method by acetylating *α*-pericyclocamphane with glacial acetic acid and sulphuric acid and then saponifying the acetylated product as shown in the following scheme:



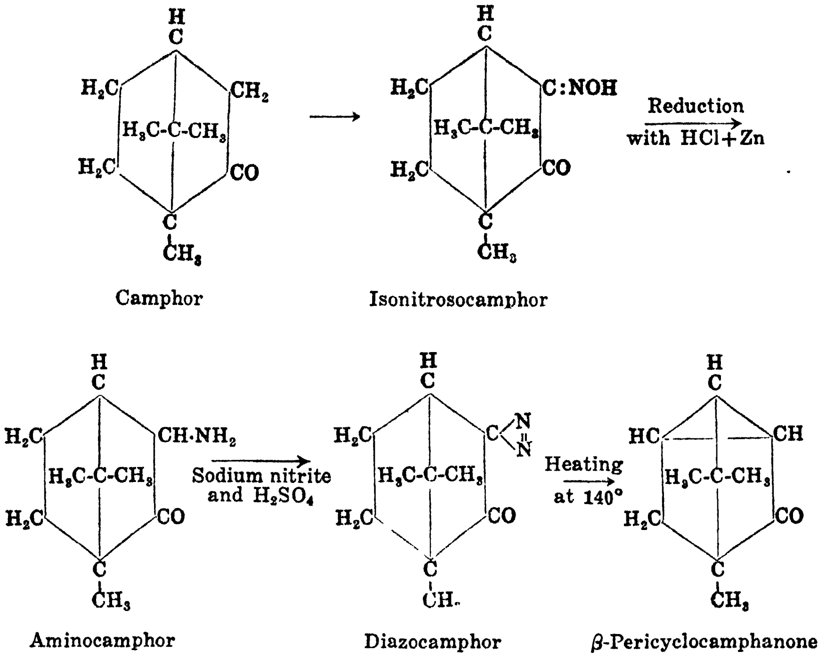
α-Pericyclocamphane

Acetyl-isoborneol

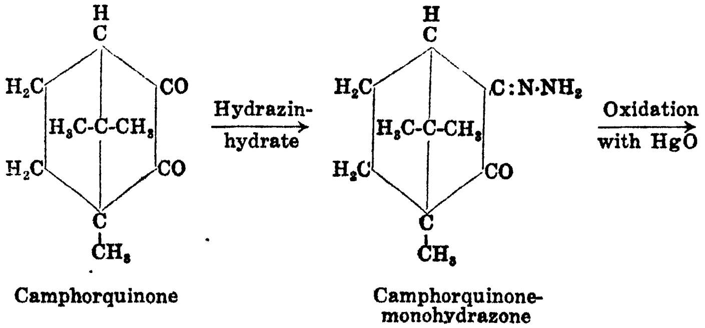
Isoborneol

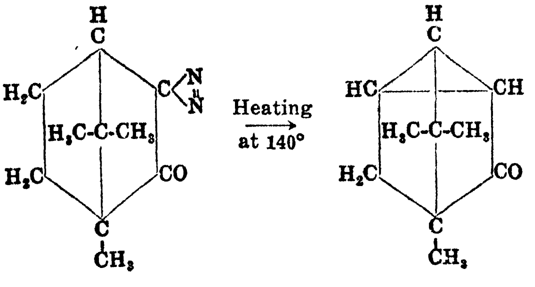
Applying this method to *β*-pericyclocamphanone; and using trichloracetic acid in place of glacial acetic acid, the present author succeeded in synthesizing 5-oxy-camphor which was identical with the one isolated from dog's urine according to the method of Asahina and co-workers. The starting material, β-pericyclocamphanone, was prepared by heating diazocamphor at 140°C. There are two methods for the preparation of diazocamphor: one is amino camphor-method,(4) and the other is camphorhydrazone-method.(5) These methods are briefly stated in the following schemes.

[A] Aminocamphor-method.



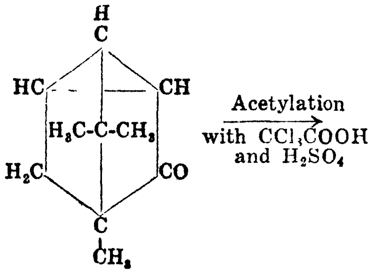
[B] Camphorhydrazone method.



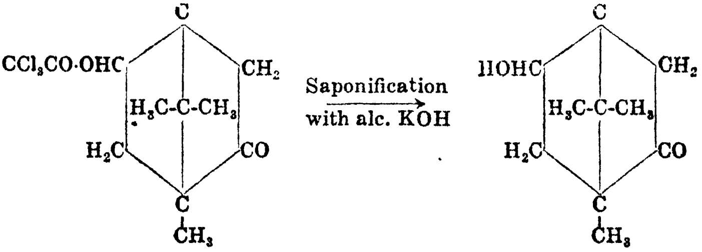


Diazocamphor β-Pericyclocamphanone

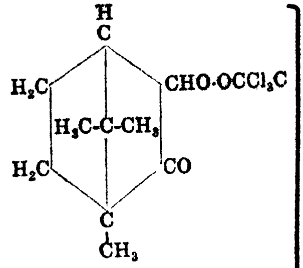
5-Oxy-camphor from β-pericyclocamphanone was prepared as follows:



β-Pericyclocamphanone



5-Oxy-camphor-trichloracetic acid ester 5-Oxy-camphor



3-Oxy-camphor-trichloracetic acid ester

Experimental.

Part I. *Preparation of β-Pericyclocamphanone.*

A. Aminocamphor-method.

(1) Isonitrosocamphor.

Five hundred grams of commercial camphor (m.p. = 176∼7°C, $[\alpha]_D^{18} = +46.99°$ in abs. alc. c = 7.08) was dissolved in 1250 c.c. dry ether in a flask, to which 74 g metallic sodium was added. Cooling the flask with ice at 10∼15°C, dry methyl nitrite gas (which was generated by dropping a solution of 230 g sodium nitrite in 500 c.c. water into the mixture of 400 c.c. water, 120 c.c. methyl alcohol, and 90 c.c. concentrated sulphuric acid) was passed into the flask. After several hours, the etherial solution was shaken with water and the aqueous solution of the sodium isonitrosocamphor was washed with ether to remove borneol and the excess of camphor, and then neutralized with dilute hydrochloric acid. Isonitrosocamphor thus obtained was recrystallized from dilute methyl alcohol; prismatic crystals; yield: 167 g.

m. p. = 151∼152°C (uncorr.)
$[\alpha]_D^{24} = +200°$ in abs. alc. (c = 3.90)

(2) Aminocamphor sulphate.

One hundred grams of isonitrosocamphor was dissolved in 1,000 c.c. 50% hydrochloric acid and reduced with 200 g zinc dust. Sufficient sodium hydroxide solution was added and the oily aminocamphor separated thereby was then dissolved in ether and added with dilute sulphuric acid drop by drop until the aqueous solution became slightly acid. On evaporating the solution, aminocamphor sulphate separated out as white lustrous crystals. Yield: about 65 g;

m. p. = 213∼214°C (uncorr.)
$[\alpha]_D^{18} = +9.51°$ in water (c = 4.51)

(3) Diazocamphor.

By adding sodium nitrite and dilute sulphuric acid into the aqueous solution of aminocamphor sulphate, cooling by means of ice, diazocamphor separated out as yellow crystals. It was then purified from petroleum ether. Yield: 78 g pure diazocamphor from 210 g aminocamphor sulphate;

m. p.=74~75°C (uncorr.)
Analysis: 4.293 mg subs. gave 0.5880 c.c. N_2 (746.5 mm, 22°C)
N=15.57%
Calc. for $C_{10}H_{14}ON_2$　N=15.73%

(4) β-Pericyclocamphanone.

β-Pericyclocamphanone was obtained by heating, a small portion at a time, the mixture of 10 g finely powdered crystals of diazocamphor and 30 g dried copper powder in a retort at 140°C. It was purified by sublimation and subsequently recrystallized from petroleum ether. Yield: 8.5 g;

m. p.=167°C (uncorr.)
$[\alpha]_D^{18}=+63.35°$ in abs. alc. (c=1.61)
Analysis: 3.710 mg subs. gave 10.785 mg CO_2, 3.120 mg H_2O;
C=79.28%　H=9.40%
Calc. for $C_{10}H_{14}O$　C=79.92%　H=9.42%

B. Camphorhydrazone-method.

(1) Camphormonohydrazone.

Twelve grams of hydrazine hydrate was gradually added, small portion at a time, into the boiling alcoholic (40 c.c.) solution of camphorquinone (25 g) (Kahlbaum's preparation, m. p.=195~196°C, $[\alpha]_D^{20}$ −80.82° in benzol, c=1.69). The hydrazone separated on cooling was recrystallized from alcohol. Yield: 20.3 g;

m. p.=198~199°C (uncorr.)
Analysis: 4.716 mg subs. gave 0.6331 c.c. N_2 (762 mm, 19°C)
N=15.73%
Calc. for $C_{10}H_{16}ON_2$　N=15.55%

(2) Diazocamphor.

Thirteen and eight-tenths grams of camphormonohydrazone was dissolved in 100 c.c. hot benzol and then added 22 g yellow mercuric oxide, and the mixture was heated on a water bath for ten hours. Diazocamphor thus separated out was purified from petroleum ether. Yield: 10.3 g:

m. p.=72°C (uncoor.)
Analysis: 2.642 mg subs. gave 0.3420 c.c. N_2 (763 mm, 13.2°C)
N=15.50%
Calc. for $C_{10}H_{14}ON_2$　N=15.73%

(3) β-Pericyclocamphanone.

β-Pericyclocamphanone was prepared from the diazocamphor in the same manner as described in the previous experiment [A]. (4);

m. p. = 167°C
$[\alpha]_D^{17} = +63.37°$ in abs. alc. ($c = 3.66$)
Analysis: 2.849 mg subs. gave 8.240 mg CO_2, 2.357 mg H_2O;
C=78.9% H=9.26%
Calc. for $C_{10}C_{14}O$ C=79.92% H=9.42%

Part II. *Preparation of 5-Oxy-camphor.*

(1) Acetylation product of β-pericyclocamphanone.

Five grams of β-pericyclocamphanone, 20 g trichloracetic acid and 0.5 g concentrated sulphuric acid were mixed in a flask provided with air-cooler, and heated in oil-bath at 150～160°C for ten hours. After cooling, the resinous mass was extracted with ether and the etherial solution was successively washed with water, with 5% sodium carbonate solution, and again with water. After evaporating the ether off, the residue was subjected to steam distillation in order to remove the excess of β-pericyclocamphanone. The residue was then extracted with ether, and the etherial solution was treated with animal charcoal, and succesively dehydrated, evaporated and distilled. The distillate thus obtained became pasty on keeping in ice-box for several days. Yield: 1 g;

b. p. = 4 mm 140～145°C (uncorr.)
$[\alpha]_D^{17} = +9.30°$ in abs. alc. ($c = 3.44$)
Analysis: 3.606 mg subs. gave 6.270 mg CO_2, 1.736 mg H_2O;
C=47.42% H=5.3%
Calc. for $C_{12}H_{15}O_3Cl_3$ C=45.93% H=4.7%
10.34 mg subs. gave 12.43 mg AgCl;
9.97 〃 〃 〃 12.16 〃 〃
Cl=29.74%
〃 =30.17%
Calc. for $C_{12}H_{15}O_3Cl_3$ Cl=33.9%

Trichloracetic acid ester of 5-oxy-camphor from dog's urine was prepared by the same way as mentioned above.

One half gram f 5-oxy-camphor and 1 g trichloracetic acid were placed in a flask with air-cooler, and heated in oil-bath at 150°C for two hours. The reaction product was then subjected to steam distillation and the residue was extracted with ether, washed with animal charcoal. After dehydration and evaporation, it was kept in a vacuum desiccator for several days, and analyzed as follows;

$[\alpha]_D^{16} = +26.60°$ in abs. alc. ($c = 1.84$)
Analysis: 10.18 mg subs. gave 12.38 mg AgCl
10.06 〃 〃 〃 12.22 〃 〃
Cl=30.09%
〃 =30.03%
Calc. for $C_{12}H_{15}O_3Cl_3$ Cl=33.39%

(2) 5-Oxy-camphor.

Two grams of acetylation product of β-pericyclocamphanone mentioned above were dissolved in 94% alcohol containing 5 g potassium hydroxide, and heated in a flask, fitted with reverted cooler, on water-bath for two hours. After cooling, the mixture was neutralized by passing CO_2 gas and then distilled to remove the alcohol. The residue was then extracted with ether, and after washing the etherial solution with water, it was dehydrated and evaporated. On recrystallization from hot ligroin (b. p.=80~120°C), it formed nice characteristic crystals (Photo. 2) which were colourless, odourless, and adhesive as camphor; hardly soluble in cold ligroin, but easily soluble in alcohol, ether, benzol and acetic acid. Yield: 0.5 g;

m. p.=222~222.5°C (uncorr.)
$[\alpha]_D^{17} = +42.7°$ in abs. alc. ($c=0.34$)
$[\alpha]_D^{18} = +43.5°$ in abs. alc. ($c=2.09$)

Mixed with the specimen of 5-oxy-camphor (m. p.=222°C, $[\alpha]_D^{24} = +43.9$(6) Photo. 1) prepared from dog's urine, no depression of melting point was observed.

Molecular weight (freezing point depression method);

0.1286 g subs. in 33.3 g benzol $\Delta=0.11°$ mol. wt.=170.5
Calc. for $C_{10}H_{16}O_2$··············· ······mol. wt.=168
Analysis: 3.440 g subs. gave 8.989 mg CO_2, 2.934 mg H_2O;
$C=71.27\%$ $H=9.54\%$
Calc. for $C_{10}H_{16}O_2$ $C=71.4\%$ $H=9.5\%$

Semicarbazone of the above synthetic 5-oxy-camphor was prepared in the usual way;

m. p.=232~3°C
Analysis: 3.979 mg subs. gave 0.6497 c.c. N_2 (753 mm, 15.0°C)
$N=19.18\%$
Calc. for $C_{11}H_{19}O_2N_3$ $N=18.6\%$

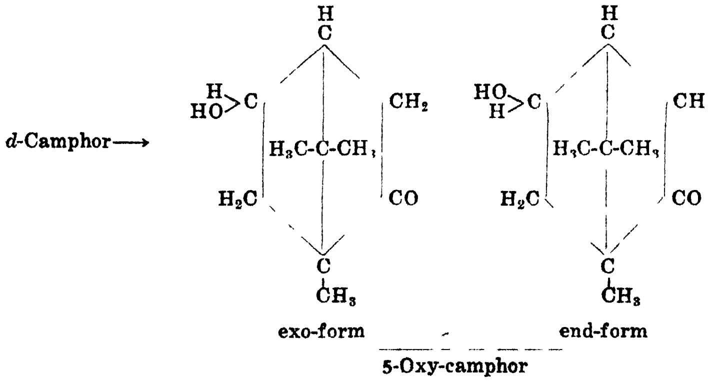
Acetyl-5-oxy-camphor was prepared by heating 1 g of the above 5-oxy-camphor and 1 c.c. acetic anhydride on oil-bath at 140°C for two hours. The excess of acetic anhydride was decomposed with water, and the reaction product was extracted with ether. The etherial solution was washed with 5% sodium carbonate solution, evaporated and distilled;

b. p.=23 mm 145~147°C (uncorr.)
$[\alpha]_D^{19} = +26.27$ in abs. alc. ($c=1.18$)
Analysis: 4.079 mg subs. gave 10.105 mg CO_2, 3.036 mg H_2O;
$C=67.57\%$ $H=8.33\%$
Calc. for $C_{12}H_{18}O_3$ $C=68.6\%$ $H=8.6\%$

The preparation of synthetic 5-oxy-camphor agreed with that obtained from dog's urine by Y. Asahina and M. Ishidate as shown in the table below:

	Y. Asahina and M. Ishidate	The present author
5-Oxy-camphor	m. p. = 222° (217~8°)C $[\alpha]_D^{20}$ = +43.2°(41.3°) in alc. C=71.04% H=9.6%	m. p. = 222~222.5°C $[\alpha]_D^{18°}$ = +42.7~43.5° in alc. C=71.27% H=9.54%
5-Oxy camphor-semicarbazone	m. p. = 233~5°C N=19.1%	m. p. = 232~3°C N=19.1%
Acetyl-5-oxy-camphor	b.p.=27 mm 159~60°C $[\alpha]_D^{20}$ = +24.8 in alc. C=68.58% H=9.00%	b. p.=23 mm 145~7°C $[\alpha]_D^{19}$ = +26.27 in alc. C=67.57% H=8.33%

After all, starting with *d*-camphor, the author has succeded in synthesizing 5-oxy-camphor which was identical both in physical and chemical properties with the preparation isolated from dog's urine. Theoretically, however, two stereoisomers of 5-oxy-camphor can be produced from *d*-camphor as shown in the following scheme:



Further investigations on this subject are still being carried on and the reports will be published in near future.

The author whishes to express his sincere thanks to Professors U. Suzuki and T. Yabuta for their kind guidances throughout this work, and also to Dr. Y. Sahashi for his kihd advices. For analyses, the author is indebted to Mr. S. Funahashi and Miss S. Matsumoto.

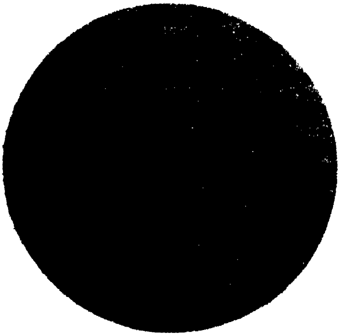


Photo. 1—5-Oxy-camphor from dog's urine.

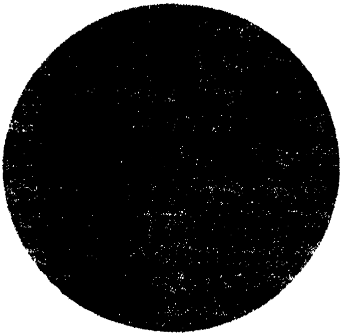


Photo. 2—Synthetic 5-oxy-camphor.

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Studies on Amylosynthease.

By

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I. Introduction.

The existence of an enzyme in the autolysed extract of brewer's yeast which has the power of polymerising achroodextrines into higher ones was first observed by S. Nishimura⁽¹⁾ in 1930 and the name "Amylosynthease" was given to it. The present author continued the studies on this subject, at first in association with the above named author and afterwards independently and succeeded in preparing the enzyme quite free from amylase, which occurs always associated with the former and interferes the polymerisation process, exerting the reverse reaction.

From the studies on the chemical nature of this enzyme, behaviors towards various reagents, optimum temperature and pH value for its activities etc., the author has come to the conclusion that amylosynthease is a new enzyme quite different from amylase and the both exert no reversible reaction.

It was observed that this enzyme is widely distributed in many varieties of yeasts and other microbes, as well as in higher plants.

The author has further proved that trihexosan and hexahexosan, prepared according to the method of A. Pictet, by heating starch with glycerine at 200~210°C can be polymerised by amylosynthease and has isolated hexahexosan from the polymerisation products of trihexosan. This gives strong evidence that the enzyme has synthetic power.

It was further observed that the dextrines prepared by the action of various amylolytic enzymes upon starch can be generally polymerised to the higher ones giving blue colouration with iodine, while the lower dextrines obtained by the action of mineral acids upon starch can never be regenerated though they seem to be identical with the products obtained by enzymes. This provides a new means of distinguishing the dextrines prepared by different methods.

The author has also determined the physico-chemical constants of polymerisation products or the socalled "synthetic starches", such as viscosity, saponification value, acetyl value, rotatory power etc. and found close resemblance with those of natural soluble starches. The X-Ray spectrogram and photometric curves have also great similarity with natural ones. But, at present, there is no means of settling the question whether the synthetic products are really identical with natural ones.

Finally, the author has demonstrated the existence of a complement of amylosynthease in the yeast extract.

In the following is given a brief summary of the results reported by the author in the Journal of Agricultural Chemical Society of Japan(2) and also in the proceedings of the Imperial Academy of Japan.(3)

II. Isolation of amylosynthease.

(1) Preparation of yeast extract.

Fresh beer yeast was well washed with water, pressed between the filter papers and dried in a vacuum desiccator. 10 g of the dried yeast thus obtained were introduced into the flask, with 100 c.c. water. After adding 10 c.c. toluol the flask was vigorously shaken and kept for two days at 25~26°C. The extract was now filtered through paper and the clear filtrate itself was used for the experiment.

(2) Soluble starch solution: Soluble starch was prepared by treating

the starch of glutinous rice with cold 7.5% hydrochloric acid solution. After removing the acid by suction, it was extracted with boiling water and filtered. The filtrate thus obtained gave a red colour with iodine and could be used directly for the experiment. In some cases, however, the soluble starch was isolated from the above filtrate by precipitating with strong alcohol.

(3) The polymerisation by amylosynthease was demonstrated in the following experiment. 2 liters of a 1% clear solution of soluble starch (pH 6.2) were introduced in a large erlenmeyer flask with 100 c.c. of the yeast extract prepared as above mentioned. After adding 7 drops of chloroform to prevent the bacterial growth, the flask was stoppered with cork plug and kept in a thermostat at 25~26°C. The clear solution became soon opaculent and a white flocculent precipitate was formed, which gradually grew larger until at last a very voluminous mass of the precipitate separated out.

The original solution, giving a red colour with iodine, became after 6 hours violet in colour, which turned into bluish violet more and more as the time went on. After standing for a week, the precipitate was collected on a filter, washed with alcohol and ether, and dried. The yield was about 84 % of the original soluble starch used. The white amorphous powder thus obtained dissolved in hot water almost completely, but precipitated out again on cooling. It gives a bluish violet colour with iodine.

(4) The control soluble starch solution, without any addition of the yeast extract, but otherwise treated in the same way as above, remained unchanged for a month and not even any slight turbidity was produced in it and when treated with iodine, the solution gave only a red colour.

(5) Isolation of amylosynthease from yeast extract.

The clear yeast extract was saturated with ammonium sulphate, whereby a flocculent precipitate was produced, which carried down a greater part of amylosynthease. This precipitate was collected on a filter and freed from the mother liquid by suction, washed with a little cold water to remove the adhering ammonium sulphate, washed again with ether and dried in a vacuum desiccator.

The yellowish white powder thus obtained was still contaminated with a little amylase. Now the author has observed that amylase is easily soluble in glycerine, while amylosynthease is not. Thus, by washing the above preparation thoroughly with glycerine, the amylase was completely removed. The amylosynthease thus prepared was found to be very active.

(6) Fractional precipitation of amylase and amylosynthease.

When 100 c.c. of yeast extract was treated with 28~30 g ammonium sulphate, a flocculent precipitate was formed, which carried down a greater part of amylase.

The filtrate therefrom gave when saturated with ammonium sulphate, a second precipitate retaining chiefly amylosynthease, which may be further purified by washing with glycerine.

The separation of the two enzymes was thus easily effected.

The glycerine extract of the first precipitate had strong amylolytic power and acted on the boiled potato starch very quickly until the solution gave no colouration with iodine.

(7) Precipitation of amylosynthease by lead acetate.

When lead acetate was added to the yeast extract until it reached about 1% of the solution, a white flocculent precipitate was formed, including a greater part of amylosynthease and leaving amylase in solution.

This precipitate was now collected on a filter by suction and after washing with cold water, it was suspended in water and treated with a small quantity of sodium bicarbonate, while passing CO_2 gas in it, whereby the amylosynthease was dissolved out in the solution and the filtrate therefrom exhibited a strong polymerising power upon soluble starch.

(8) Isolation of amylosynthease from potato tubers.

Fresh potato was crushed to a fine pulp, adding the same volume of a 1/30 mol. phosphate buffer solution (pH 8,0), filtered through cloth and then through paper. The filtrate thus obtained was now saturated with ammonium sulphate, whereby a flocculent precipitate was formed, including both amylase and amylosynthease. By thoroughly washing the precipitate with glycerine, however, the amylase could be removed completely and the insoluble residue retained only amylosynthease.

(9) Isolation of amylosynthease from polished glutinous rice.

Polished glutinous rice is finely powdered, macerated with 3~4 volumes of cold water, filtered through cloth and then through paper. When the filtrate thus obtained is treated with 1/100~1/160 mol. solution of cadmium chlōride, a yellowish white precipitate is formed, which carries down amylosynthease leaving amylase in solution. The precipitate is now suspended in water and saturated with ammonium sulphate, whereby an active amylosynthease is obtained.

III. General properties of amylosynthease.

(1) Elementary composition of amylosynthease.

The amylosynthease precipitated by cadmium chloride from the extract of beer yeast, or baker's yeast (Fleischmann's yeast) or from the extract of glutinous rice and purified by treating with ammonium sulphate, following by washing with glycerine is a white amorphous powder, easily soluble in cold water and gives biuret, xanthoprotein and Molisch's reaction.

They contain in average 8% nitrogen, 30% carbon, 5% hydrogen, 28% cadmium chloride and 5% ash.

The existence of a little sulphur and phosphorous is also detected.

(2) Behaviours toward various reagents.

The amylosynthease is completely precipitated from its aqueous solution by adding lead acetate or cadmium chloride and can be regenerated from the precipitate by the methods above mentioned. The amylase is thereby left in solution.

The amylosynthease is also precipitated by a little mercuric chloride, but it can never be regenerated from this precipitate by any treatment, while amylase inactivated by the same reagent can be easily regenerated by treating the precipitate with hydrogen sulphide. Thus, we see that there is a remarkable difference between amylase and amylosynthease in the behavior towards various reagents and the latter must be considered to be a new enzyme quite different from amylase, though it occures always associated with the latter.

Further, amylosynthease is completely thrown down from its aqueous solution by adding a little lead nitrate, ferric nitrate, ferric chloride, cadmium nitrate, aluminium nitrate or aluminium chloride.

When the precipitates, thus obtained are suspended in a little cold water and saturated with ammonium sulphate, white insoluble residues are obtained, which retain an active enzyme.

The amylosynthease is also precipitated by silver nitrate, copper nitrate, copper chloride, copper acetate, mercuric acetate, platinium chloride and zinc chloride. But, it can never be regenerated from these precipitates by any treatment.

Cobalt chloride, nickle chloride, magnesium chloride and barium chloride have no power of precipitating the enzyme from the solution.

The precipitate of amylosynthease formed by the addition of mangan chloride, ammonium alum and feather alum behaves as such like the enzyme itself, when added to the solution of soluble starch.

Thus, we see that the behavior of various metalic salts upon amylosynthease is quite defferent according to the nature of metals.

(3) Protective action against chemical reagents.

Toluol has no inhibiting action, while chloroform behaves noxiously upon amylosynthease. The noxious effect of the latter, however, can be protected by dextrine. Thus, when a solution of dextrine is treated with a little amylosynthease and after adding a few drops of chloroform, stoppered with cork plug, slightly shaken and kept in a thermostat at 20~25°C, the polymerisation proceeds very quickly.

Formaldehyde, carbon disulphide and carbon tetrachloride behave also harmfully like chloroform.

(4) The amylosynthease is precipitated by alcohol and aceton, and at the same time becomes insoluble in water. The precipitate obtained by saturating with ammonium sulphate is easily soluble, but it can not be precipitated again by the same reagent.

(5) Action of pancreatic tryptase on amylosynthease.

When 1~2% aqueous solution of pancreatine (Japanese Pharmacopoea) is added with ammonium sulphate up to 24~25%, a white flocculent precipitate is obtaind. This precipitate is collected on a filter, dissolved in 30 times of water after drying in vacuum and treated with aceton up to 80%, whereby tryptase is again precipitated. By repeating this process, tryptase quite free from amylase can be obtained.

This pancreatic tryptase has no action upon amylosynthease, so it is presumed that amylosynthease is not of protein nature, though it is precipitated by ammonium sulphate in its impure state.

(6) Adsorption of amylosynthease.

The amylosynthease is adsorbed by aluminium hydroxyde, aluminium phosphate, monocalcium phosphate, caolin and animal charcoal. From these adsorbants, the enzyme can be again dissolved out by treating with a dilute sodium bicarbonate solution.

Amylase is not adsorbed by caolin, so it can be easily separated from amylosynthease,

(7) Optimum temperature and opt. pH value.

The amylosynthease is very sensitive to high temperature, when the yeast extract is preserved at 30°C, the activity is nearly lost in a day, while it is quite stable at lower temperature (0~15°C) and may be kept for a month without loosing much in activity. In dry state, however it can be preserved at room temperature for several months.

The opt. temperature is estimated to be 20~25°C; it is completely destroyed by heating at 50°C for 10 minutes.

The opt. pH value of yeast amylosynthease is 6.2 and that of potato and rice is 6.8~7.0.

IV. Distribution of amylosynthease.

Amylosynthease seems to be widely distributed in various kinds of yeasts, thus it is detected in beer-, wine-, distiller's and baker's yeasts, as well as in saccharomyces ellepsoideus, s. biwa, s. eryobotoryae, s. apiculatus, film forming yeast and mycoderma; but, it is absent in saccharomyces saké, s. pasteurianus, zigos. major, willia anomala, pichia menbranaceae, tolura A., oidium lactis, botrytis, aspergilus oryzae, bac. subtilus, bac. coli, and acetic bacteria.

Its presence is also confirmed in potato, common and glutinous rice.

V. Polymerisation of trihexosan and hexahexosan.

The author has prepared trihexosan and hexahexosan according to the method of A. Pictet by heating starch with glycerine at 200～210°C and confirmed that these two compounds can be polymerised into higher ones by the action of amylosynthease, thus, the aqueous solution of trihexosan which gave no colouration with iodine, become after adding a little amylosynthease, red, violet and finally bluish violet. Hexahexosan which gives red colour with iodine behaved also in the same manner.

The author has further isolated hexahexosan from the polymerisation products of trihexosan. For this purpose, the aqueous solution of trihexosan was treated with a little amylosynthease and when the iodine reaction became red, the solution was evaporated and poured into a large quantity of alcohol and the precipitate thus formed was subjected to fractional precipitation with alcohol. In this way, hexahexosan was isolated and was proved to be identical with the product obtained by the method of A. Pictet.

VI. Polymerisation of glycogen and dextrine prepared from different origins by different methods.

(1) Polymerisation of glycogen.

The glycogen used in this experiment was prepared by digesting commercialy purified glycogen with cold 7.5% hydrochloric acid and precipitating with alcohol after 2 or 3 days. It dissolved clearly in water and gave a brownish red colour with iodine. Now, by adding a little amylosynthease to the above solution, the polymerisation proceeded at once and the colour gradually changed to red, violet and finally to blue.

It is interesting to note that genuine glycogen which can scarcely be acted by amylosynthease, becomes easily polymerisable after treating with cold hydrochloric acid as above stated.

The degradation products of glycogen, obtained by the action of amylase can also be polymerised.

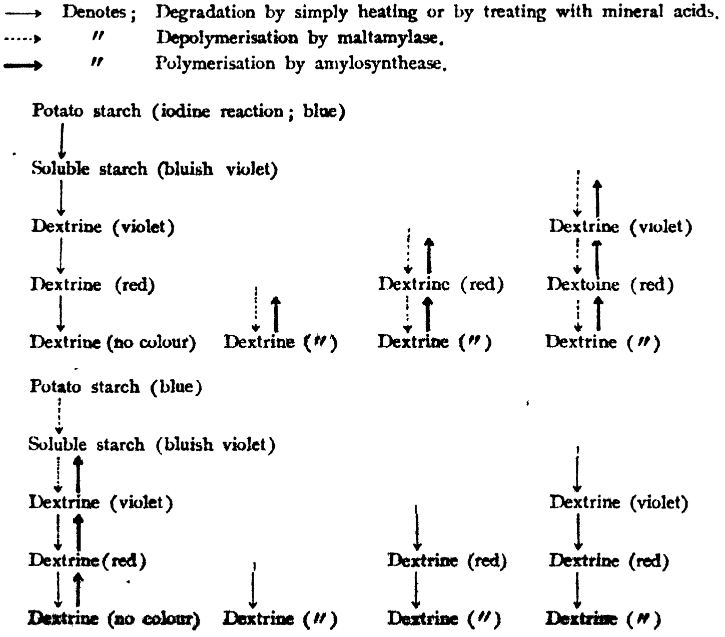
(2) Polymerisation of dextrines contained in various grains.

Natural dextrines seem to be widely distributed in various kinds of grains. For the preparation of it, finely powdered grain is boiled with 95% alcohol and the insoluble residue is extracted with cold water. The dextrines thus obtained give generally red or brown colour with iodine, but sometimes the reaction is negative. All these preparations are proved to be polymerisable by amylosynthease. Soluble starches prepared from glutinous millet (*Panicum miliaceum*) and foxtail millet (*Setalica italica*) by treating with cold 7.5% hydrochloric acid are also polymerised by amylosynthease and form insoluble precipitates with closely resemble to the products obtained from rice dextrine. Further, the dextrines and soluble starches were acetylated and purified by decolorizing with animal charcoal and by precipitating again with alcohol. When the acetylated products thus obtained were saponified with alcoholic potash to remove acetyl groups and the regenerated dextrines were treated with amylosynthease the polymerisation proceeded at the same rate as before acetylation. It was observed, however that the dextrines, except "grenzdextrine", have undergone certain changes, because the degradation products of them could never be polymerised again by amylosynthease. Similarly the degradation products obtained by the action of mineral acids upon dextrines are not polymerisable.

(3) Polymerisation of dextrines prepared by various methods.

It was observed that the dextrine prepared from potato starch by maltamylase can be easily polymerised by amylosynthease, while those prepared by simply heating or by treating with mineral acids can never be polymerised. It seems thus these dextrines are chemically different, though the both give red coloration with iodine.

Further, various kinds of dextrines were prepared and their behavior toward amlosynthease was observed with the following results:



It can be seen that in the above experiments, chemically different dextrines are always produced according to the method of preparation.

The author has prepared dextrines, which give no colouration with iodine, i. e. β-glucosidomaltose (R. Ling) and amylotriose (H. Pringsheim) by the methods above mentioned and found that it was impossible to distinguish them by chemical or physical means, but the behavior toward amylosynthease was quite different, i. e. β-glucosidomaltose could be polymerised, while amylotriose not.

As the reducing power of dextrine disappears after polymerisation, it is presumed that free aldehyde group is responsible for the polymerisation and in the case of trihexosan and hexahexosan, free aldehyde group might be formed instanteniously in the molecule during polymerisation.

VII. Relative velocity of polymerisation of different dextrines.

With the purpose of determining the relative velocity of polymerisation of different dextrines, each 1% solution of soluble starch and amylose of glutinous rice, achroodextrine, erythrodextrine and hexahexosan was prepared and treated with the amylosynthease solution (prepared by macerating 150 g dry yeast with 900 c.c. water) at room temperature and the times required until the solution give bluish violet colour with iodine were estimated. The results are graphically shown in Fig. 1.

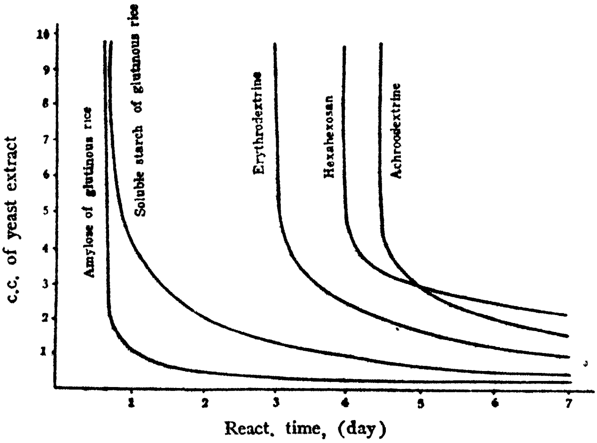


Fig. 1. 25 c.c. of 1% solution (hexahexosan, achroodextrine, erythrodextrine, amylose or soluble starch of glutinous rice) is added with yeast extract (at room temperature).

From these results, it can be seen that the dextrines of larger molecules can be more quickly polymerised than those of smaller molecules.

In Fig. 2 the activity of amylosynthease previously kept in aqueous solution at different temperature is graphically described.

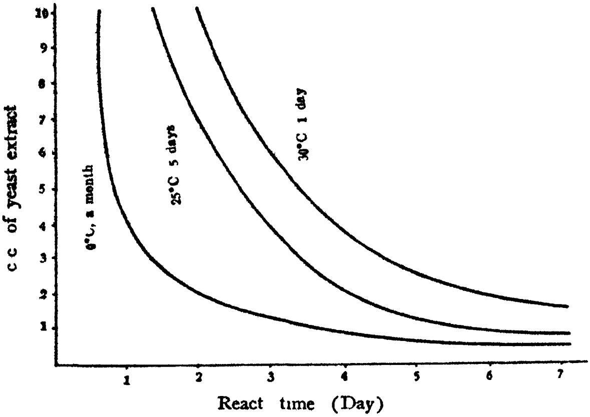
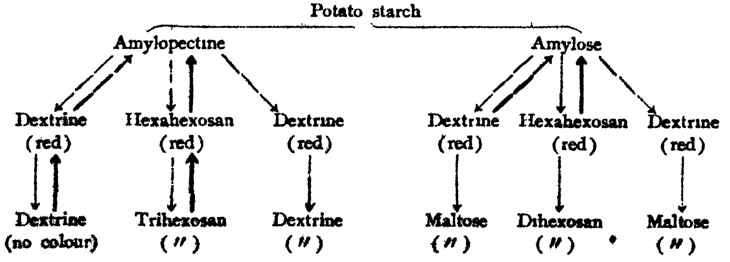


Fig 2 The yeast extracts previously kept for 1 day at 30°C, for 5 days at 25°C and for a month at 0°C resp , were added each to 25 c c. of 1% solution of soluble starch of glutinous rice (at roomtemperature)

VIII. Polymerisation of the derivatives of amylose and amylopectine.

(1) Amylose and amylopectine were treated either by maltamylase, by heating with glycerine or by treating with conc. hydrochloric acid and the behaviors of these decomposition products toward amylosynthease were observed with the following results:

→ Denotes; Depolymerisation by maltamylase.
⇢ 〃 Decomposition by heating with glycerine.
⤏ 〃 Degradation by treating with mineral acids.
⟶ 〃 Polymerisation by amylosynthease



By such a means, it is possible to determine whether the chemical configuration of certain carbohydrates are analogous to amylose or to amylopectine.

(2) On lichen starch (Isolichenin).

Isolichenin is widely distributed in many varieties of japanese mooses, i. e. in alectoria ochrolenca, pertusalia trocophota, ramalina genuculata, spharophorus collaloidrs, thamnolia vermicularis, usnea trichodea, cetralia islandica, etc..

It forms a white amorphous powder, clearly soluble in hot water and gives blue coloration with iodine. It is interesting to note that isolichenin (from alectoria ochrolenca) is gradually decomposed by maltamylase giving blue, violet, red and finally yellow coloration with iodine just like starch.

The behaviors of these decomposition products toward amylosynthease were compared with those of glycogen and it was confirmed that the configuration of isolichenin is analogous to amylose and that of glycogen to amylopectine.

IX. Physico-chemical properties of synthetic starches.

4 kinds of synthetic starches were prepared by amylosynthease from different sources, i. e, from soluble starches of glutinous rice and millets (A), from dextrine prepared by maltamylase (B), from hexahexosan (C) and from soluble glycogen (D), and their general composition, saponification value, viscosity, rotatory power as well as the acetyl value were estimated.

It was confirmed that these synthetic products have great similarity with the natural soluble starches.

Further, it was shown that the preparation (B) and (D) give typical crystalline X-Ray spectrogram and photometric curves which closely resemble to those of natural starches (Fig. 4).

X. On the complement of amylosynthease.

The yeast extract is treated with 70% alcohol and the precipitate thereby formed is further purified by dissolving in water, filtering through paper and again precipitating with 70% alcohol. In this way, a white powder is obtained which is easily soluble in cold water and posseses the power of accelerating the activity of amylosynthease, as is shown in Fig. 3.

When the filtrate of the above precipitate is concentrated in vacuum and treated with 95% alcohol, an yellowish white precipitate is obtained, which has strong accelerating power upon amylase.

Further, it was observed that glutathion and cystein which strongly accelerate amylase have no action upon amylosynthease as shown in the

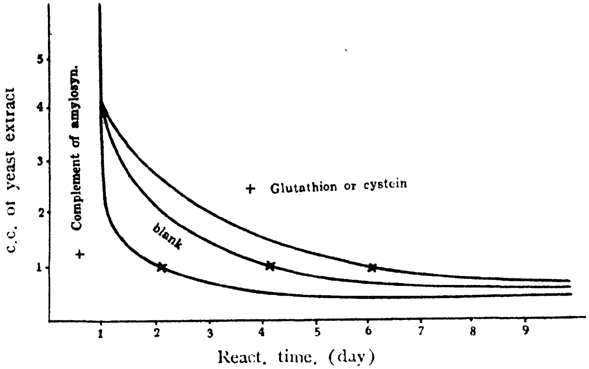


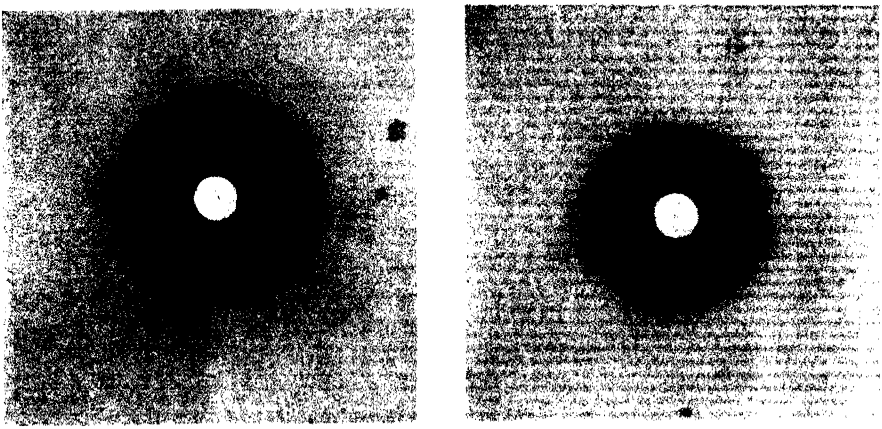
Fig. 3. 25 c.c. of 1% solution of soluble starch of glutinous rice is added with yeast extract, complement of amylosynthease + yeast extract or glutathion or cystein + yeast extract.

following experiment:

Soluble starch from glutinous rice treated with:	Iodine reaction after:	
	1 day	2 days
Amylosynthease from glutinous rice	red violet	violet
〃 + Complement	violet	bluish violet
〃 + glutathion or cystein	brown	yellow

The above result shows that glutathion or cystein exerted rather inhibiting action upon amylosynthease.

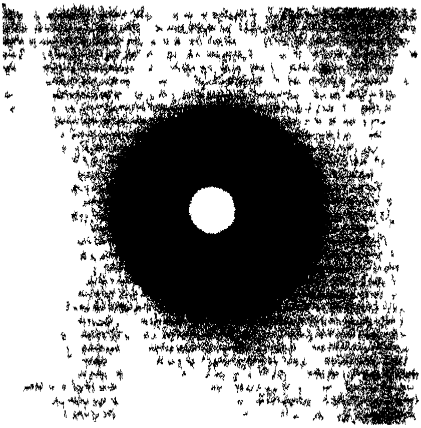
Fig. 4. Diffraction figure



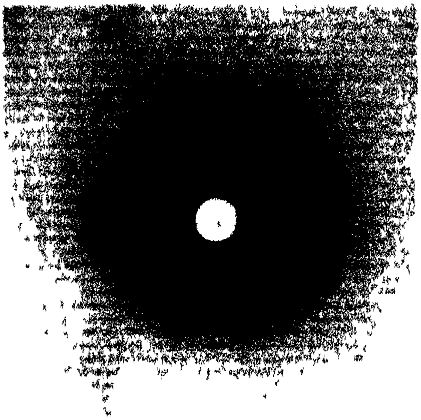
No. 1 (Soluble starch)

No. 2 (Synthetic starch B.)

No 3 (Potato starch)

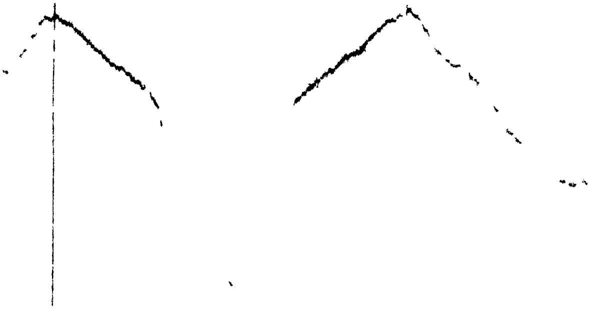


No 4 (Synthetic starch D)



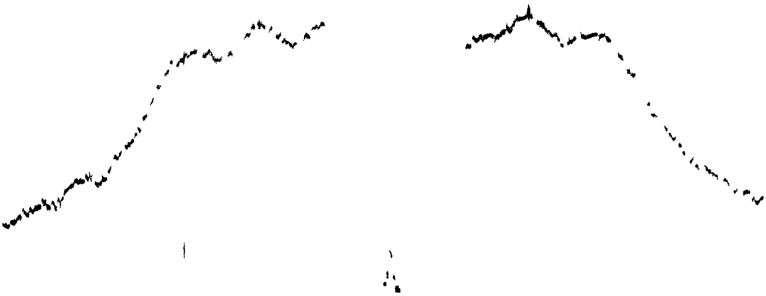
Photometric curve

No 1 (Soluble starch)



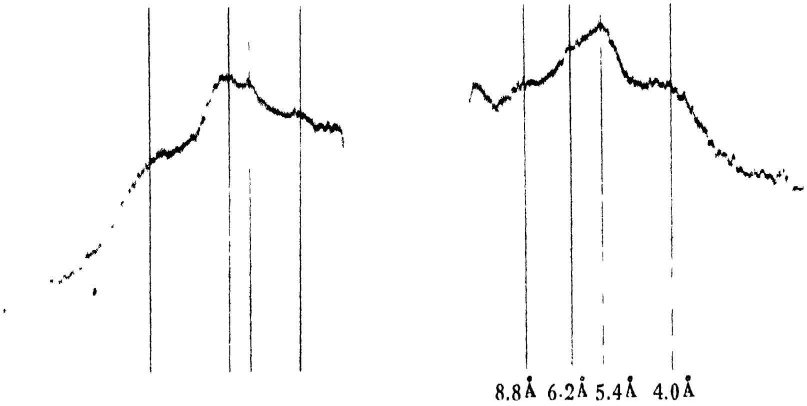
55 Å 4 0 Å

No 2 (Synthetic starch B)

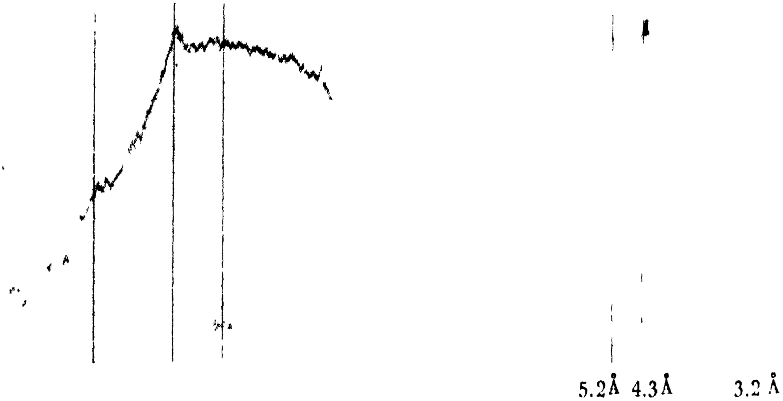


7 1 Å 4 6 Å

No. 3 (Potato starch)



No. 4 (Synthetic starch D.)



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On the Glycogen Content in the Liver of Pigeons and Rats by B_1-Avitaminosis.

By

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(Received February 21, 1934.)

Introduction.

Many authors such as Funk and Schönborn(1), Ogata(2), Collazo(3), Bickel and Collazo(4) etc. have observed that the liver glycogen of pigeons and fowls suffering from vitamin B-deficiency is always less than the normal healthy ones, sometimes being reduced to the minimum Quite different result was, however, obtained by Abderhalden and Wertheimer(5), who fed pigeons with polished rice or with synthetic B-free diets and observed an enormous increase of glycogen in the liver and heart of the diseased animals but by giving dried yeast or B_1-preparation, the glycogen content is soon reduced to the normal level. It seems thus that B_1 plays an important rôle for the metabolism of glycogen in the liver. The present experiment was undertaken to contribute more data for the decision of this problem.

Experimental.

(1) Liver Glycogen of Pigeons fed on Polished Rice.

Pigeons weighing about 300 g were divided into two groups and the 1st. (Control) group, consisting of 9 pigeons, was fed on unpolished rice ad. libitum, each bird being kept in a separate wire cage.

The glycogen content in the liver was estimated according to the Pflüger's method modified by Laurence and McCance(6) with the following results:

Table 1. Liver Glycogen of Pigeons fed on Unpolished Rice (Control).

Pigeon No.	Body weight			Liver glycogen. (%)	No. of days fed.
	At the biginning of feeding. (g)	At the end of feeding. (g)	Increase (+) or decrease (—) in weight. (%)		
1	315	321	+ 1.9	2.01	4
2	326	339	+ 4.0	3.49	4
3	328	331	+ 0.9	3.42	4
4	338	344	+ 1.8	2 82	6
5	328	362	+10.4	4.76	6
6	279	272	— 2.5	2.92	6
7	274	294	+ 7.3	2.78	6
8	277	278	+ 0.4	3.29	8
9	288	287	+ 0.3	3.97	9
Ave.				3.28	

The 2nd. group, consisting of 18 pigeons, was fed on polished rice supplemented daily with 0.06 g McCollum and Simmonds salt mixture and 0.4 g autoclaved yeast as the source of B_2. The symptoms of B_1-deficiency were roughly classified into three types, i. e. spastic-, paralytic-and atrophic forms. At the day or the following, when the definite symptom of polyneuritis was developed, 14 pigeons were killed and the liver glycogen was estimated, while the remaining ones were cured by administering each 4 mg active oryzanin (B_1-preparation of Sankyo and Co.) and after complete recovery, the liver glycogen was estimated.

Table 2. Liver Glycogen of Pigeons fed on Polished Rice.

Pigeon No.	Body weight.			Liver glycogen. (%)	Days elapsed before the appearance of the disease.	Symptoms.
	At the beginning of feeding. (%)	At the appearance of the disease. (%)	Decrease in weight. (%)			
1	229	203	−32.1	6.35	23	Spas.
2	339	205	−39.5	6.25	26	Par.
3	335	213	−36.4	5.98	21	″
4	292	213	−27.1	4.58	36	Spas.
5	336	222	−33.9	6.77	26	Par.
6	332	210	−36.7	3.75	32	″
Ave.: Pigeons weighing over 200 g.…………………………				5.46		
7	321	175	−45.5	0.86	40	″
8	330	198	−40.0	+	39	″
9	298	161	−46.0	+	26	″
10	270	151	−44.0	2.17	32	″
11	325	170	−47.7	0.82	32	″
12	306	188	−38.6	1.17	19	Spas.
13	299	145	−51.5	0	22	Par.
14	351	170	−51.3	0	26	Atr.
Ave.: Pigeons weighing below 200 g. ………………………				1.26		
15	307	226	−26.4	3.78	16 (4;−3)	Spas.
16	310	223	−28.1	2.37	23 (11;−4)	″
17	303	189	−37.6	2.82	20 (9;+3)	Par.
18	316	176	−44.3	2.88	50 (9;−1)	″
Ave.: Pigeons cured by B_1-preparation.……… …………………				2.96		

Figures in parenthesis indicate No. of days treated with active oryzanin and the increase or decrease of body weight is denoted with (+) or (−).

It can be seen from the above table that there is a great difference in the glycogen content among the pigeons, though they were equally fed on polished rice, namely, those which developed the disease without loosing much in body weight contained larger quantity of glycogen than those which have heavily lost in body weight due to the loss of apetite at the later period of the experiment and suffered from severe inantion. Thus the average content in pigeons weighing over 200 g was 5.46%, while those weighing less than 200 g was only 1.26%. Most probably, the glycogen once deposited in large quantity was again used up during the stage of inanition. The pigeons cured by the administration of B_1-preparation had in average 2.96% glycogen. This is nearly the same with that of the control group fed on unpolished rice, i. e. 3.28%.

(2) Liver Glycogen of Pigeons fed on B_1-Free Synthetic Diet.

Pigeons weighing about 300 g were divided into two groups and the 1st. group, consisting of 17 pigeons, was fed on a synthetic diet, deficient in B_1 but otherwise adequate for growth.

It consisted of: Fish meat protein 15, starch 72, butter fat 10, McCollum and Simmonds salt mixture 3. Fish meat protein and starch were purified by extracting with alcohol and ether. Butter fat was treated with hot water and alcohol. As the source of B_2, 0.4 g autoclaved yeast were given daily. The 2nd. (Control) group was fed on the same diet as above except that the autoclaved yeast was substituted with the same amount of dried yeast. The results were as follows:

Table 3. Liver Glycogen of Pigeons fed on B_1-Free Synthetic Diet.

Pigeon No.	Body weight.		Decreare in weight. (%)	Liver glycogen. (%)	Days elapsed before the appearance of the disease.	Symptoms.
	At the beginning of feeding. (g)	At the appearance of the disease. (g)				
1	280	250	−10.7	6.93	29	Spas.
2	260	206	−20.8	6.14	26	〃
3	308	246	−22.1	5.52	27	〃
4	335	255	−23.9	6.81	13	〃
5	336	233	−30.7	4.93	36	Par.
6	328	215	−34.4	6.43	32	Spas.
7	321	200	−37.7	6.55	30	〃
8	329	200	−39.2	5.47	38	〃
Ave.: Pigeons weighing over 200 g.				6.10		
9	277	175	−36.8	3.59	33	Par.
10	283	175	−38.1	0.45	34	〃
11	311	172	−44.7	1.29	29	〃
12	305	176	−45.6	0.90	42	〃
13	316	128	−59.5	1.33	35·	Atr.
Ave.: Pigeons weighing below 200 g.				1.51		
14	302	224	−26.1	2.46	23 (5; + 5)	Spas.
15	285	201	−29.5	2.13	19 (8; +14)	Par.
16	273	211	−22.7	1.63	16 (11; ± 0)	Spas.
17	320	227	−29.1	2.50	23 (6; + 9)	〃
Ave.: Pigeons cured by B_1-preparation.				2.18		

Table 4. Liver Glycogen of Pigeons fed on the Complete Synthetic Diet (Control).

Pigeon. No.	Body weight.			Liver glycogen. (%)	No. of days fed.
	At the beginning of feeding. (g)	At the end of feeding. (g)	Increase (+) or decrease (−) in weight. (%)		
1	272	268	−1.5	1.90	9
2	272	272	±0.0	2.36	9
3	300	295	−1.7	1.86	11
4	312	295	−5.4	1.47	16
Ave.				1.90	

The above results agree well with that of the 1st. experiment fed on rice diet, i. e. those pigeons weighing over 200 g had in average 6.10% and those weighing less than 200 g contained only 1.51%, while those cured by B_1 had nearly the same amount as that of control ones, i e. 2.18% against 1.90%.

(3) Liver Glycogen of Underfed Pigeons.

In this experiment, pigeons were fed on restricted amount of unpolished rice together with 0.4 g dried yeast and when the loss of body weight attained 20%∼30%, the glycogen was estimated with the following results:

Table 5. Liver Glycogen of Underfed Pigeons.

Pigeon No.	Body weight.			Liver glycogen. (%)	No. of days fed.
	At the beginning of feeding. (g)	At the end of feeding. (g)	Decrease in weight. (%)		
1	289	240	−17.0	1.62	8
2	321	258	−19.6	+	11
3	304	238	−21.7	2.61	13
4	295	227	−23.1	4.00	10
5	256	183	−24.6	1.26	13
6	282	212	−25.0	2.05	15
7	256	192	−25.0	1.81	13
Ave.: Pigeons which have lost 20% body weight.				2.23	
8	300	219	−27.0	1.15	15
9	304	218	−28.3	2.13	15
10	273	194	−28.9	3.41	13
11	316	221	−30.1	0	15
12	298	208	−30.2	0.35	10
13	335	202	−39.7	0	10
Ave.: Pigeons which have lost 30% body weight.				1.76	

As the above table indicates, the glycogen decreases at any stages of inanition and so it can be said that the mere inanition is quite different from polyneuritis as to the effect on the glycogen content in the liver.

(4) Liver Glycogen of Albino Rats fed on B_1-Free Synthetic Diet.

The typical symptom of polyneuritis was more frequently manifested by albino rats than by pigeons when fed on the following B_1-free synthetic diet: Fish meat protein 18, starch 68, butter fat 10, McCollum and Simmonds salt mixture 4. To this diet, 0.4 g autoclaved yeat were added. Young male rats weighing about 60 g were kept each in a separate wire cage and fed with the above diet and when the animals developed the disease, a part of them was cured by the administration of 4 mg active oryzanin daily. For control animals, 0.4 g dried yeast were supplemented to the B_1-free diet above mentioned.

Table 6. Liver Glycogen of Rats fed on B_1-Free Synthetic Diet.

Rat No.	Body weight. At the beginning of feeding. (g)	Body weight. At the appearance of the disease. (g)	Increase (+) or decrease (−) in weight. (%)	Liver glycogen. (%)	Days elapsed before the appearance of the disease.	Symptoms.
1	56.0	42.5	−24.1	4.66	32	Spas.
2	61.5	58.5	− 4.9	8.02	33	〃
3	65.0	51.0	−21.5	8.04	34	〃
4	60.0	69.0	+15.0	6.13	36	〃
5	59.0	58.0	− 1.7	7.94	47	〃
6	56.5	54.0	− 3.5	6.96	37	〃
Ave.				**6.96**		
7	55.0	55.5	+ 0.9	3.65	31 (15;+17.0)	〃
8	56.0	56.0	± 0.0	3.82	33 (14;+ 8.5)	〃
9	57.0	55.5	− 2.6	1.82	32 (13;+17.5)	〃
Ave.: Rats cured by B_1-preparation.				**3.10**		

Table 7. Liver Glycogen of Rats fed on the Complete Synthetic Diet (Control).

Rat No.	Body weight. At the beginning of feeding. (g)	Body weight. At the end of feeding. (g)	Increase in weight. (%)	Liver glycogen. (%)	No. of days fed.
1	**61.0**	74.5	+22.5	4.96	**14**
2	**56.0**	80.5	+65.2	5.50	**14**
3	**62.0**	70.5	+13.7	5.08	**14**
4	**62.0**	97.5	+57.3	3.50	**14**
5	**63.0**	66.5	+ 5.6	2.88	**15**
6	**67.0**	106.0	+58.2	4.03	**13**
Ave.				**4.33**	

(5) Liver Glycogen of Underfed Albino Rats.

Rats were fed with the restricted amount of the basal diet above mentioned, supplemented with dried yeast and the glycogen content was estimated with the following results:

Table 8. Liver Glycogen of Underfed Rats.

Rat No.	Body weight. At the beginning of feeding. (g)	At the end of feeding. (g)	Decrease in weight. (%)	Liver glycogen. (%)	No. of days fed
1	65.0	57.5	−11.5	0.84	9
2	69.0	60.0	−13.7	1.77	10
3	70.0	60.0	−14.3	0	9
4	72.0	61.0	−15.3	0	15
5	77.5	65.0	−16.1	3.31	13
6	65.0	54.5	−16.2	0	15
7	75.5	62.0	−17.9	1.88	13
8	69.0	55.0	−20.0	+	19
9	80.0	63.5	−20.6	2.88	13
Ave.: Rats which have lost 10~20% body weight.				2.14	
10	65.5	47.5	−27.5	0.67	21
11	63.5	45.5	−28.5	0.77	21
12	71.0	50.0	−29.6	+	15
13	71.0	49.0	−34.0	0	19
Ave.: Rats which have lost 30% body weight.				0.72	

The above result is in full agreement with the 3rd. experiment on pigeons, i. e. those which have lost 30% of the original body weight contained only 0.72% in average.

(6) Liver Glycogen of Albino Rats fed on High-Fat Diets lacking both B_1 and Carbohydrates.

For the control, a complete high-fat diet was prepared as follows: Fish meat protein 29, lard 45, butter fat 20, McCollum and Simmonds salt mixture 6. To this, 0.4 g dried yeast were added daily.

The glycogen content was as follows:

Table 9. Liver Glycogen of Rats fed on the Complete High-Fat Diet (Control).

Rat No.	Body weight. At the beginning of feeding. (g)	At the end of feeding. (g)	Increase in weight. (%)	Liver glycogen. (%)	No. of days fed
1	57.5	104.5	+ 81.7	1.72	87
2	60.0	144.0	+140.0	2.16	87
3	58.5	129.5	+121.4	2.03	65
4	60.0	124.0	+106.7	1.82	65
Ave.				1.92	

The composition of B$_1$-free, high-fat diet used in this experiment was the same with the control diet except that 0.4 g autoclaved yeast were used instead of dried yeast. The animals fed on this diet developed typical avitaminosis more slowly and the liver glycogen was far less than those fed on the carbohydrate rich, B$_1$-free diet as shown in the following table:

Table 10. Liver Glycogen of Rats fed on B$_1$-Free, High-Fat Diet.

Rat No.	Body weight			Liver glycogen. (%)	Days elapsed before the appearance of the disease.	Symptoms.
	At the beginning of feeding (g)	At the appearance of the disease (g)	Increase (+) or decrease (−) in weight. (%)			
1	59.5	79.5	+33.6	1.91	32	Spas.
2	59.5	83.5	+40.5	2.39	70	〃
3	66.0	90.0	+36.4	1.66	65	〃
4	60.0	66.0	+10.8	1.40	66	〃
5	58.0	51.0	−12.1	1.70	35	〃
6	55.5	49.5	−10.8	1.58	47	〃
Ave.:				1.77		
7	60.5	53.0	−12.4	1.47	74 (6;+ 2.0)	〃
8	67.0	69.5	+ 3.7	1.31	38 (16;+22.0)	〃
9	61.5	53.5	−13.0	1.43	41 (13;+ 9.5)	〃
10	42.5	33.5	−21.2	2.09	31 (7;+11.5)	〃
Ave.: Rats cured by B$_1$-preparation.				1.75		

In the next experiment, the above high-fat, B$_1$-free diet was supplemented with absolute ethyl alcohol and saccharose respectively to the extent of 10%. In this case also the animals developed the disease but both alcohol and saccharose had apparenly no noticeable effect upon the glycogen content in the liver as shown in the following tables:

Table 11. Liver Glycogen of Rats fed on B$_1$-Free, High-Fat Diet added with 10% Alcohol.

Rat No.	Body weight			Liver glycogen. (%)	Days elapsed before the appearance of the disease.	Symptoms.
	At the beginning of feeding. (g)	At the appearance of the disease. (g)	Increase (+) or decrease (−) in weight. (%)			
1	62.5	54.0	−13.6	1.20	63	Spas.
2	67.5	91.5	+35.5	1.15	71	〃
3	56.5	87.0	+54.0	1.61	75	〃
4	67.0	74.5	+11.2	1.46	77	〃
Ave.				1.34		

Table 11. Continued.

5	63.5	53.0	−16.5	1.60	42 (7 ; +17.5)	〃
6	61.0	66.5	+ 8.2	2.66	53 (7 ; + 8.5)	〃
7	63.5	73.0	+15.0	2.05	47 (12 ; +12.0)	〃
8	70.5	72.0	+ 2.1	2.19	67 (6 ; +16.5)	〃
9	58.0	80.0	+38.0	1.94	55 (14 ; +13.5)	〃
10	61.0	51.5	−15.6	1.19	74 (8 ; + 9.5)	〃

Ave. : Rats cured by B_1-preparation. ………………………… 1.94

Table 12. Liver Glycogen of Rats fed on B_1-Free, High-Fat Diet added with 10% Saccharose.

Rat No.	Body weight. At the beginning of feeding. (g)	At the appearance of the disease. (g)	Increase (+) or decrease (−) in weight. (%)	Liver glycogen. (%)	Days elapsed before the appearance of the disease.	Symptoms.
1	63.0	49.0	−22.2	1.73	32	Spas.
2	59.0	52.5	−11.0	†	33	〃
3	59.0	60.0	+ 1.7	1.99	54	〃
4	59.0	56.0	− 4.2	1.74	42	〃
5	62.5	45.5	−27.2	+	30	〃
Ave.				1.82		
6	60.5	69.0	+14.5	1.77	32 (9 ; +21.0)	〃
7	59.0	58.0	− 1.7	2.08	32 (9 ; +28.0)	〃
8	59.5	57.5	− 3.4	1.70	36 (9 ; +20.0)	〃

Ave. : Rats cured by B_1-preparation. ………………… 1.85

Summary.

(1) The glycogen contents in the liver of pigeons and albino rats suffering from vitamin B_1-deficiency were estimated.

(2) In the case of pigeons, those which developed the disease without loosing much in body weight stored up more glycogen than the normal ones, i. e. 1.5 to 2.0 times higher than the latter, while those suffered from severe inanition and consequently heavily loss in body weight had always less glycogen than the control. In pigeons cured by the administration of active oryzanin (B_1-preparation), the liver glycogen was nearly normal.

(3) The underfed birds had always less glycogen than the normal ones.

(4) In albino rats fed on carbohydrate rich synthetic diet and developed the disease, the liver glycogen was without exception 1.5 times higher than the normal ones. In the animals cured by active oryzanin, the glycogen content was again reduced to the normal level.

(5) Though the rats fed on B_1-free, high-fat diet lacking carbohydrates have shown the typical symptom of avitaminosis, yet there was no increase of glycogen in the liver.

(6) It may be assumed that the abnormal increase of glycogen in the liver of avitaminous animals fed on B_1-free diet is a secondary phenomenon of the disease, but not the cause of it.

(7) As the diets used in these experiments contained B_2 in the form of autoclaved yeast, it can be concluded that B_1 has played an important rôle for the metabolism of liver glycogen.

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Untersuchungen über die Enzyme von Bombyx mori L. VI. Mitteilung.

Über die Blutlipase.

Von

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(Eingegangen am 5, Januar 1934)

Der erste Versuch zu einer quantitativen Bestimmung der Blutlipase bei hoheren Tieren findet sich in der Arbeit von Rona und Michaelis⁽¹⁾, die die Spaltung von Mono- oder Tributyrin durch das Ferment mit der stalagmometrischen Methode verfolgten.

Was Insekten betrifft, so ist vielleicht zum erstenmal vom Verf. ein solcher Versuch gemacht worden und im nachstehenden mochte er die Ergebnisse seiner Untersuchungen über die Blutlipase von Bombyx mori mitteilen.

1) *Methodik*:- Wenn nicht anders angegeben, wurden 25 ccm gesattigte wasserige Tributyrinlosung, 2.5 ccm M/3 Phosphatpufferlosung von Ph 6.8 und 0.5 ccm Blut vermischt. Je nach 0, 10, 20 und 30 Minuten Erwarmung bei 20° wurde die Abspaltung von Tributyrin mit dem Stalagmometer ermittelt. Die Wirksamkeit der Blutlipase wurde mit den Konstanten, die nach der Gleichung der monomolekularen Reaktion berechnet wurden, verglichen.

2) *Optimale Ph.*

Larve, ♀		Larve, ♂		Puppe, ♀	
Ph	$k.10^2$	Ph	$k.10^2$	Ph	$k.10^2$
5.3	0.48	6.5	0.53	7.0	0.83
6.2	0.67	6.8	0.98	7.4	0.85
7.0	1.20	7.2	1.12	7.7	0.96
8.0	1.18	7.7	1.49	8.0	0.73

3) *Optimaltemperatur.*

Larve, ♀, Ph=6.8		Puppe, ♂, Ph=7.7	
Temp.	$k.10^2$	Temp.	$k.10^2$
5	0.56	20	0.33
20	1.23	30	0.77
35	3.46	40	1.16
50	3.07	50	1.05

4) *Kinetik.*

Bei Puppe: 50 ccm gesätt. Tributyrinlösung + 1.5 ccm Pufferlösung + 0.5 ccm Blut, Ph = 7.7, Temp. = 20°.

Zeit, Minuten	Larve, ♀, Ph=6.8		Puppe, ♀, Ph=7.7	
	a−x	$k.10^2$	a−x	$k.10^2$
0	73	——	73	——
10	47	1.91	60	0.85
20	31	1.86	50	0.82
30	20	1.87	41	0.84
40	—	——	34	0.83
50	—	——	28	0.83
60	—	——	24	0.81

a−x bedeutet die in der Zeit t noch vorhandene Menge der Tributyrins (gesättigte wässerige Lösung = 100).

Puppe, ♀, Ph=6.8		Puppe, ♂, Ph=7.7	
Fermentmenge, ccm	$k.10^2$	Fermentmenge, ccm	$k.10^2$
0.25	0.47	0.20	0.48
0.50	0.88	0.40	0.91

5) *Einfluss von Chinin oder Atoxyl.*

2 ccm Pufferlösung + 0.5 ccm Blut + 1 ccm Giftlösung. Nach 30 Min. Zugabe von 50 ccm Tributyrinlösung.

♀, Ph=7.7		♂, Ph=6.8		♀, Ph,=7.7		♀, Ph=6.8	
Chinin, mg	$k.10^2$	Chinin, mg	$k.10^2$	Atoxyl, mg	$k.10^2$	Atoxyl. mg	$k.10^2$
0	0.79	0	0.72	0	0.43	0	0.95
5.0	0.58	2.5	0.64	5.0	0.38	2.5	0.64
10.0	0.41	5.0	0.53	10.0	0.36	5.0	0 55

6) *Unterschied zwischen gut gewachsenen und schlecht gewachsenen Raupen.*

	Gut gewachs. Raupen		Schlecht gew. Raupen	
	♀	♂	♀	♂
$k.10^2$	1.11	0.99	1.14	1.06

7) *Änderungen durch Hunger bei der Larve.*

	Hungerstund.	1	23	47
$k.10^2$	♀	0.72	0.84	1.27
	♂	1.09	1.06	1.40

8) *Unterschied zwischen gesunden und kranken Raupen.*

	Gesunde Raupen		Krank Raup. (Nankabyo)	
	♀	♂	♀	♂
$k.10^2$	1.52	1.06	1.58	1.36

9) *Veränderungen im Laufe der drei Entwicklungsperioden von Bombyx mori.*

			$k.10^2$	
	Tage		♀	♂
Larve	V. Lebensalter	3	0.86	0.93
		6	0.72	1.09
		8	1.14	1.15
Einspinnen des Kokons	2		1.89	2.19
	5		2.00	1.76
Puppe	1		1.70	1.71
	3		1.41	1.55
	7		0.95	1.18
	12		0.82	0.60
Schmetterling	1		0.79	—

10) *Diskussion der Versuchsresultate.*

Wie oben erwahnt, habe ich in meiner vorliegenden Arbeit über einige Eigenschaften der Blutlipase von Bombyx mori geforscht und aufgeklart, dass diese Lipase in ihrer enzymatischen Natur derjenigen des Blutes höherer Tiere sehr nahe steht.

Aber über die Herkunft der Blutlipase bei der Seidenraupe können wir wie bei höheren Tieren nichts sagen, denn wir haben keine eingehenden Angaben über die Organlipasen bei diesem Insekt.

Darauffolgend kommt die Frage der biologischen Bedeutung der Blutlipase.

Bei den höheren Tieren scheint die Blutlipase nichts mit der normalen

Ernährung zu tun zu haben. Aber in der fünften Mitteilung(2) habe ich bereits über die engen Beziehungen zwischen den Blutdisaccharasen bei der Seidenraupe und den chemischen Umsetzungen der Disacharosen im Körper derselben gesprochen. Diese Beziehung der Blutfermente von Bombyx mori zum Stoffwechsel behalten auch für die Blutlipase ihre Geltung.

Das Maulbeerblatt enthält kein echtes Fett, dagegen die Seidenraupe immer beträchtliche Mengen Körperfett aufzuweisen hat. Im Digestionssafte der Seidenraupe kommt keine oder nur wenig Lipase vor, im Blut derselben indessen eine verhältnismässig wirksame Lipase. Bei Hunger ist die Blutlipase vermehrt. Die Lipase der kranken Raupe ist wirksamer als die der gesunden. Eine erhebliche Anlagerung des Körperfetts geschieht im fünften Lebensalter der Larve. Die Puppe und der Schmetterling leben hauptsächlich auf eigene Kosten des Körperfettes. Im Laufe von drei Entwicklungsperioden von Bombyx mori wirkt die Blutlipase in den Tagen des Kokonspinnens und des Verpuppens am aktivesten.

Aus den oben angeführten Data geht hervor, dass die Blutlipase bei Bombyx mori in der Assimilation oder beim Abbau des Fetts eine wesentliche Rolle spielt.

Zusammenfassung.

(1) Die Spaltung des Tributyrins durch die Blutlipase von Bombyx mori kann in guter Annäherung mit Hilfe der Gleichung der monomolekularen Reaktion ausgedrückt werden.

(2) Die Blutlipase zeigt die optimale Aktivitat bei Ph 7.7; ihr Temperaturoptimum liegt bei 40°.

(3) Die Lipase ist gegen Chinin oder Atoxyl wenig empfindlich.

(4) Die Wirkung der Lipase ist bei kranken Raupen (Nankabyosan) viel höher als bei gesunden und auch bei schlecht gewachsenen Raupen etwas stärker als bei gut gewachsenen. Durch Hunger wird sie gesteigert.

(5) Die Blutlipase wird im fünften Lebensalter der Larve mit dem Wachstum stärker, die ihr Maximum in den Tagen des Kokonspinnens und des Verpuppens erreicht und dann ist sie in den Perioden der Puppenruhe und des Schmetterlingslebens allmählich vermindert. Es besteht kein wesentlicher Geschlechtsunterschied in der Lipasewirksamkeit.

(6) Bei Bombyx mori scheint die Blutlipase am Fettstoffwechsel Anteil zu nehmen.

Zum Schluss danke ich herzlich Herrn Prof. Y. Okuda für seine Anregung zu diesen drei Arbeiten.

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Nutritive Value of Canavanin (amino acid)

By

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(Received February 1, 1934)

A new amino acid called Canavanin was discovered and named by Dr. Kitagawa of Fukuoka Imperial University in 1929. But none has been investigated on the nutritive value of that amino acid yet. Therefore the author tried to determined the nutritive value of that amino acid.

In this experiment, the author employed a number of young male albino rats weighing about 42 grams. Divided the animals into 4 groups, and fed them on a diet deficient in Canavanin. To the group I, gave Canavanin 0.1 gram per Kilo-gram of the body weight per day. To the group II, gave Canavanin 0.05 gram per Kg. of the body weight per day, and to the group III, gave Canavanin 0.01 gram per Kg. of the body weight per day. But to the fourth group, did not give any Canavanin, on account of this group was a control.

The author fed the animals as mentioned in the above way for 40 days, and obtained the following results.

1. The mortality of the animals fed on Canavanin was rather lower than the animals which was not administered Canavanin. Therefore Canavanin is not poisonous or harmful substance.

2. The animals administered Conavanin had a flow of spirits, while the animals which did not received Canavanin were rather depression of spirits.

3. The animals administered Canavanin were grew better and increased the body weight more rapidly than the animals which did not consume Canavanin.

4. Regard to the amount of the consumption of diet, the animals administered Canavanin was smaller amount than the animals which were not administered Canavanin.

Researches on the Electrolytic Reduction Potentials of Organic Compounds. Part 18.

On the electrolytic reduction of camphor, again.

By

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Abstract from original paper.

(Recived March 1, 1934)

The polarographic study of camphor was previously performed by Prof. M. Shikata and M. Watanabe. The authors found that the polarograms of camphor had generally two reduction waves.

Their reduction potentials were independent on hydrogen-ion concentration of electrolyzing solutions and their saturation currents were much smaller than other various compound in same condition.

The present author studied on the electrolytic reduction of camphor again to explain the mechanism of the appearance of abnormal reduction waves.

He found that three waves appeared on the polarogram obtained from 0.1 n HCl containg 10^{-2} m camphor.

He discussed the phenomena after cansideration of electrocapillar curve and concluded as follows.

(1) The first wave is not due to the true reduction but due to the replacement of Cl′ to camphor molecule on the electrode surface.

(2) The second wave is the true reduction wave, which potential shifted by the change of pH as expected by Nernst's formula.

(3) The third wave is due to the destroy of molecular camphor on the electrode surface.

Hochsiedende unverseifbare Substanz des Fuselöls (II. Mitteilung)

Von

T. Taira und T. Masuzima.

(Eingegangen am 1, März 1934)

Der eine der Verf. (T. Taira)(1) hat in der vorigen Mitteilung darauf hingewiesen, dass er aus dem hochsiedenden Anteil des Rohrzuckermelassefuselöls Methylheptylcarbinol isoliert und im Ipomoeafuselöl ausser den beiden vermuteten sekundären Alkoholen Phenyläthylalkohol nachgewiesen hat. Ferner hat er Phenyläthylalkohol im Reisbranntweinfuselöl und Sake isoliert und festgestellt. Auch hat er(2) vor mehreren Jahren mitgeteilt, dass er aus dem hochsiedenden unverseifbaren Anteil des Reisbranntweinfuselöls sesquiterpen- und diterpenartige Kohlenwasserstoffe isolieren konnte. Die vorliegende Untersuchung befasst sich mit den hochsiedenden, unverseifbaren Anteil der Ipomoea- und Rohrzuckermelassefuselöle sowie andrer Fuselöle. (Eine eingehende diesbezügliche Mitteilung ist gegenwartig unter Druck und zwar im Journ. Agr. Chem. Soc. of Japan).

I. n-Heptylalkohol.

Faget(3) hat aus der Tatsache, dass n-Heptylsäure unter den Oxydationsprodukten des Weintreberfuselöls vorkommt, geschlossen, dass darin n-Heptylalkohol enthalten ist. Der so erhaltene Heptylalkohol vom Sdp. 155~160° ist nach ihm von dem durch Reduktion des Oenanthol gewonnenen n-Heptylalkohol vom Sdp. 175° verschieden. Später wurde Heptylalkohol von Windisch(4) im Kornfuselöl, von Claudon und Morin, von Ordonneau(6), von Ost(7) im alten Kognak vesmutet. Auch in Japan vermuteten einige Autoren,(8)(9)(10) dass n-Heptylalkohol in einigen Fuselölen und im Reisbranntwein enthalten ist. T. Taira hat zwar in der vorigen Mitteilug darauf(1) hingewiesen, dass er beim Nachweis der sekundären Alkohol im Rohrzuckermelassefuselöl den primären Alkohol nicht finden konnte, doch haben diesmal Verf. zur Entscheidung der Frage, ob n-Heptylalkohol enthalten ist oder nicht, die beiden folgenden Versuche angestellt:

(A) Nach Destillation des unterhalb 132° siedenden Anteils aus 54 Liter Rohrzuckermelassefuselöl unter gewöhnlichem Druck wurde der unverseifbare Teil getrennt, dann wurde Phtalsäureanhydrid auf die unter 80 mm zwischen 105~130° siedende, 64 ccm betragende Fraktion in Benzollösung einwirken lassen. Der Phtalsäureester des primären Alkohols wurde mit Alkali abgeschieden, mit überschüssigem Alkali verseift, der unverseifbare Teil mittels

Äther getrennt und der fraktionierten Destillation unterworfen, wobei nur eine geringe Menge der den Methylamylcarbinol und Phenyläthylalkohol entsprechenden Fraktion, aber kein n-Heptylalkohol erhalten wurde.

(B) Nach gleichem Verfahren wurde der hochsiedende, unverseifbare Anteil aus 21 Liter Rohrzuckermelassefuselöl vom Amylalkohol getrennt. 20 ccm der uner gewohnlichem Druck zwischen 160~180° siedenden Fraktion wurden mit Phtalsäureanhydrid behandelt und die primaren Alkohol isoliert, aber ein mit dem Siedepunkt 175° des n-Heptylalkohols übereinstimmender Anteil nicht erhalten. Nur eine sehr geringe Menge Substanz, die nach sekundärem Alkohol roch, wurde erhalten.

Der Siedepunkt des von Faget(3) zuerst isolierten Heptylalkohols stimmte mit dem des Methylamylcarbinols üeberein. Verf. vermuten, dass die von ihm erhaltene n-Heptylsäure vom Methylheptylcarbinol stammt.

II. Phenyläthylalkohol des Rohrzuckermelassefuselöls.

In der vorigen Untersuchung konnte Phenyläthylalkohol im unverseifbaren Anteil des Ipomoea- sowie Reisbranntweinfuselöls nachgewiesen werden, aber die dem Phenyläthylalkohol entsprechende Fraktion des Rohrzuckermelassefuselöls bildete zwar Diphenylharnstoff, aber Urethan konnte in befriedigender Weise nicht dargestellt werden. Die beiden Fraktionen vom Sdp. 120~130° unter 40 mm und 120~135° unter 20 mm aus 54 Liter Fuselol wurden vereinigt und nach der oben erwahnten Methode wurden mittels Phtalsaureanhydrid die primären Alkohole aus 160 ccm der Mischung isoliert, wodurch eine farblose und klare, etwas nach sekundären Alkoholen riechende, dabei einen starken Rosengeruch aufweisende Fraktion von 60 ccm mit dem Sdp. 120~126° unter 40 mm erhalten wurde. $d\frac{20°}{4°} = 1{,}0072$. Das in üblicher Weise hergestellte und aus Alkohol umkristallisierte Phenylurethan hatte den Schmp. 80° und die Mischprobe zeigte Keine Depression. Die Stickstoffbestimmung nach Dumas ergab 5,9% N; dieser Gehalt mit der berechneten Menge $(C_{15}H_{15}O_2N)$ von 5,81% fast üeberein.

III. Sesquiterpenalkohole.

(A) Sesquiterpenalkohol aus Ipomoeafuselöl.

Beim Fraktionieren des hochsiedenden, unverseifbaren Anteils des Ipomoeafuselöls wurde eine schön blaue, zwischen 140~150° unter 5 mm siedenden Fraktion in einer etwa 0,3% des Fuselöls entsprechenden Ausbeute erhalten. Dieselbe ging bei erneuter Fraktionierung unter 5 mm zwischen 143~145° über. Beim Erhitzen mit metallischem Natrium verharzte die ganze Flüssigkeit und auch bei Wiederholung der Fraktionierung verharzte ein Teil derselben. Diese wohlriechende Flüssigkeit zeigte die Liebermannsche Reaktion und entfärbte Brom. Die Analysenzahlen stimmten annähernd mit der Formel $C_{15}H_{24}O$. Durch Erhitzen mit der alkalischen Lösung der Diazobenzolsulfosäure trat eine Rotfärbung auf. Sdp./760 mm = 320°. $d^{20°}_{4°}$ = 0,9367. n_D^{20} = 1,5052. $\alpha_D = +1.8$. Mol. Refraktion: Gef. 69.61. Ber. ($C_{15}H_{24}OF_2$) = 67,67. Das Resultat wies auf eine bicyklische Verbindung hin. Durch Hydrierung bei Gegenwart von $PdCl_2$ oder Platinschwarz wurde der Wasserstoff nicht aufgenommen. Durch Bromierung nach der Methode von Allen addierte 1 Mol der verbindung 3 Atom. Brom. Weder durch Kochen der Verbindung in Benzollösung mit Phtalsäureanhydrid noch durch direktes Erhitzen mit dem letzteren auf etwa 140° trat eine Reaktion ein. Auch Phenylisocyanat reagierte nicht. Die Reaktion von Denigès auf tertiären Alkohol war positiv. Die Substanz gab keine Kristalle mit Chlorwasserstoff, Bromwasserstoff und Nitrosylchlorid. Die Dehydrierung mit Schwefel oder Selen führte nur zu Harzen und weder Eudalin noch Cadalin war nachweisbar. Durch Dehydrierung mit Ameisensäure oder Kaliumbisulfat verharzte der grösste Teil und nur geringe Mengen Kohlenwasserstoffe, deren Kp. bei etwa 260° und 300° lagen, wurden erhalten. Da dieser Sesquiterpenalkohol von der aus Rohrzuckermelassefuselöl erhaltenen Verbindung deutlich differierte, dessen Untersuchung sich Verf. Vorbehalten.

(B) Sesquiterpenalkohol aus Rohrzuckermelassefuselöl.

Auch aus Rohrzuckermelassefuselöl wurde eine mit Sesquiterpenalkohol des Ipomoeafuselöls ähnliche Fraktion in einer 1% des Fuselöls betragenden Ausbeute gewonnen. Die Farbe war nicht so tief wie beim Ipomoeafuselöl, sondern sehr schwach gelblich und unbeständig. Sdp./760 mm = 320°. $d^{20°}_{4°}$ = 0,9228. n_D^{20} = 1,5018. $\alpha_D = -1,8$. Mol. Ref: gef. 70,50; ber. ($C_{15}H_{24}OF_2$) 67,67. Dieselbe zeigte die Reaktion des tertiaren Alkohols. Bei der katalytischen Reduktion wurde der Wasserstoff nicht addiert. Bei der Bromierung nach Allen wurden 4 Atom. Brom addiert. Die Dehydrierung gab auch die gleichen Resultate wie beim Sesquiterpenalkohol des Ipomoeafuselöls d. h. eine geringe Menge zweier Kohlenwasserstoffe. Da bei der Liebermannschen Reaktion eine deutliche Verschiedenheit bemerkbar war.

IV. Diterpen.

Durch Erhitzen und Destillation der Fraktion (Sdp. 150~180° unter 5 mm) aus dem unverseifbaren Teil des Ipomoea- und Rohrzuckermelassefuselöls mit metallischem Natrium bekamen Verf. einen farblosen, klaren und fast geruchlosen Kohlenwasserstoff. Die Liebermannsche Reaktion gab keine deutliche Färbung. Der Kohlenwasserstoff entfärbte nicht Brom. Gegen Oxydationsmittel verhielt er sich beständig. Das Molekulargewicht stimmte mit Diterpen überein. Die folgende Tabelle enthält die Konstanten des Diterpen aus den beiden oben erwähnten Fuselölen und Reisbranntweinfuselöl(2):

	Ipomoeafuselöl	Rohrzuckermelassefuselöl	Reisbranntweinfuselöl
Kp./760 mm	355°	360°	355°
$d^{20°}_{4°}$	0,9310	0,9141	0,9150
$n^{20°}_{D}$	1,5170	1,5100	1,5120
α	0	0	0
Mol. Ref. gef.	88,39	88,98	89,21
〃 〃 ber.	$C_{20}H_{32}F_3$ 88,76		

V. Cetylalkohol.

Beim Abkühlen der Diterpenfraktion aus Ipomoea- und Rohrzucker melassefuselöl bildeten sich Kristallblattchen. Als Verf. die Lösung dieser Kristalle in gleichem Volumen Äthylacetat mit einer Kaltemischung genügend abkühlten, dann rasch abfiltrierten und die Substanz aus Äthylacetat umkristallisierten, erhielten sie die Verbindung vom Schmp. 49°. Dieselbe hat nach der Analyse die Zusammensetzung $C_{16}H_{34}O$. Die Mischprobe mit reinem Cetylalkohol veranlasste keine Schmelzpunktserniedrigung. Durch Einwirkung von Phenylisocyanat in Benzollosung wurden seidenglanzende Kristalle vom Schmp. 76° erhalten. Die Mischprobe mit Phenylurethan zeigte keine Depression. Nach Dumas analysiert wurde der Stickstoffgehalt 3,93% gefunden. Derselbe stimmt mit dem auf $C_{23}H_{39}NO_2$ berechneten Stickstoffgehalt 3,90% überein. In der Pflanzenwelt wurde Cetylalkohol bisher nur in Dorema ammoniak, Don gefunden, daher ist es vom grossen Interesse, dass derselbe auch im Fuselöl vorkommt.

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Studies on the Proteins Contained in Mulberry Leaves. Part IV.

A Comparative Study of the Digestibility of the Proteins Contained in Mulberry Leaves

By

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(Received April 5, 1934)

Résumé

(1) I stated in Parts II and III⁽¹⁾ that the proteins (considered collectively) contained in mulberry leaves undergo great change in quality with the growth of the leaves, or, in other words, each protein undergoes more or less change in quantity; and also that the absolute quantity of the proteins contained in the silkworm-body and in cocoon silk is always found to increase in proportion to the degrees of youthfulness of the mulberry leaves used in feeding the silkworms.

Now, here in Part IV, I have made the following studies concerning the digestibility of the proteins contained in mulberry leaves:—

(2) As the results of experimentation (described below) in artificial digestion made with the gastric juice of silkworms, I have found that the digestibility of all the proteins (considered collectively) contained in mulberry leaves varies according as the proteins change in quality with the growth of the leaves, or, to put it in plain words, the proteins contained in younger leaves are easier of digestion than the proteins contained in older ones.

The results of the experiments are as follows:—

(a) Experiments in artificial digestion with the gastric juice of silkworms have demonstrated that the protein which dissolves in the boiling 60% alcohol containing 0.3% of sodium hydroxide and which increases, with the growth of the leaves, its proportion to the total quantity of all the proteins in mulberry leaves, is less digestible than any other protein contained in the leaves and seeds of mulberry plants.

(b) When gastric juice is applied to well-crushed younger and older leaves (to which a buffer solution containing pH 9.8 has previously been applied) respectively, the digestibility of the proteins contained in the

younger leaves is found to be superior to that of the proteins contained in the older ones; while, when only the buffer solution is applied to the younger leaves, the pH value of the liquid thus obtained is found to be slightly inferior to that of a liquid similarly made from the older leaves; also, within the range of pH 9 30 to pH 9.86, which covers the scope of these experiments, it is found that the proteins contained in mulberry leaves fall in digestibility with the fall of pH values. Moreover, digestion experiments made upon each of the sample solutions that have previously been all brought to the same pH value (i. e. 9.8), also show that the digestibility of the proteins contained in the younger leaves is superior to that of the proteins contained in the older ones.

(c) I wish to add that these experiments have been made exclusively either on mulberry leaves with cell-walls well crushed, or on sap pressed out of well-crushed fresh leaves.

(3) Digestion experimets made in actual silkworm culture show the same varying degrees of digestibility observable in the proteins contained in mulberry leaves, just as in the case of the artificial digestion experiments described above. Also, the results of the silkworm culture practised in this way are found to be identical with those stated in Part III.

(4) To sum up, as the proteins contained in mulberry leaves undergo change in quality with the growth of these leaves, they also present different degrees of digestibility. In other words, the digestibility rises in proportion to the degrees of youthfulness of the leaves used in feeding the silkworms.

(5) As a result of experimentation on silkworms that have been fed on mulberry leaves in different stages of growth, it has been discovered that, when a group of silkworms have been fed on younger leaves containing a given quantity of proteins and another group on older leaves containing the same quantity of proteins, a larger proportion of the proteins contained in the younger leaves has been ingested than of the proteins contained in the older leaves. The superiority of the proteins contained in younger leaves to those in older leaves is thus proved again in this experiment, just as it was in the digestion experiments described above.

Also, the digestibility and the proportion that has been ingested of the dry matter in the leaves are found to be similar to those of the proteins contained in the leaves.

Literature.

(1) Y. Kishi: Bull. of Agricul. Chem. Soc. of Japan, Vol. IX, Nos. 1—3, 1933.

Studies on the Proteins Contained in Mulberry Leaves. Part V.

On the Quantitative Changes of All the Proteins (Considered Collectively) Contained in a Given Quantity of Fresh Mulberry Leaves, as Considered with Relation to the Growth of these Leaves.

By

Yukitaro KISHI and Yonekichi YOKOTA

(At the Katakura Research Institute of Mulberry Culture, near Hachioji City, Tokyo Prefecture, Japan)

(Received April 5, 1934)

Mésumé

(1) Here we have studied how all the proteins (considered collectively) contained in a given quantity of fresh mulberry leaves, undergo change in quantity with the growth of these leaves.

(2) As mulberry leaves come to, or near, the end of their growth due to the stems reaching an advanced stage of growth, it is found that the total quantity of all the proteins contained in a given quantity of fresh leaves becomes generally amaller than the total quantity of those in unmatured younger leaves, which is in the same way as that proteins contained in a given quantity of the dry matter in the leaves undergo change in quantity with the growth of these leaves.

However, this change in the total quantity of all the proteins contained in a given quantity of fresh leaves has been observed to be variable according to the different stages of the leaves and stems, the varieties of the plants, and other conditions of growth.

(3) One of us, who, last year, made studies concerning the proteins contained in mulberry leaves and the culture of silkworms on mulberry leaves in various stages of growth, states in Part III of these Studies that the absolute quantity of the proteins contained in the silkworm-body and in cocoon silk is always found to increase in proportion to the degrees of youthfulness of the mulberry leaves used in feeding the silkworms. This year, again, he has made an inquiry into the cause of the results mentioned in Part III, and reports the results of his experiments in Part IV, to the effect that the digestibility of the proteins contained in younger leaves is superior to that of the proteins contained in older ons, and that this digestibility rises or falls according as the proteins contained in these leaves

change in quality with the growth of the leaves.

Now, on the other hand, we wish to point out that another cause that has brought about the above-mentiond results must be that all the proteins (considered collectively) contained in fresh leaves change in quantity with the growth of the leaves, namely, that the absolute quantity of the proteins contained in the younger leaves used in feeding the silkworms is larger than that of the proteins contained in the older ones.

The writers of these pages wish to express their sincere gratitude to Professor Dr. Y. Okuda for his kind advice throughout these works (Parts IV & V).

ERRATA
to
"Studies on the Proteins Contained in Mulberry Leaves" (Part I～III), by Y. Kishi, in Vol. IX, Nos. 1～3, of this Journal.

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Isolation of Oryzanin (Antineuritic Vitamin) Third Report.

By

Sator OHDAKE.

(*Agricultural Chemical Laboratory, Faculty of Agriculture, Tokyo Imperial University, Komaba, Tokyo.*)

(Received March 29 th, 1934)

The author has recently observed that the vitamin-B_1 content of yeast can be increased considerably by suspending fresh living yeast in a solution containing vitamin-B_1 and by passing air continuously for 5~6 hrs. The material used in this experiment was prepared in this way from pressed baker's yeast and the B_1-activity was about ten times greater than common brewer's yeast.

(I) 142 kg of this material (42.790 g of dry-yeast) were extracted with water acidulated with sulphuric acid and treated with acid-clay. The activated solid thus obtained, was now extracted with baryta water. After the removal of baryta with sulphuric acid, the filtrate was concentrated to a small volume and treated with 85% alcohol to remove protein and other impurities. The alcoholic solution was then evaporated to a syrupy consistency which contained 44.2 g. of solid matter and possessed highly activity even in the dose of 0.5 mg for pigeon. This concentrate was now dissolved in 1.500 cc of water and precipitated fractionally with silver nitrate and baryta. The precipitates obtained at the Ph. 4.5~6.8 were decomposed with dilute hydrochloric acid and purified by the phosphotungstic method. The filtrate from barium phosphotungstate was freed from baryta with sulphuric acid, evaporated, then dissolved in absolute alcohol, and concentrated to a small volume after acidifying with hydrochloric acid. The resultant, mostly consisting of the crystalline hydrochloride, was dissolved in a small volume of alcohol and added cautiously with acetone until white turbidity is produced. The crystals, separated out gradually, were collected after keeping in a refrigerator and purified by repeated recrystallisation from alcohol and acetone. Yield :- 0.7 g.

(II) The same material was extracted with 60% alcohol and treated in the same manner as above. The yield was 0.15 g from 20 kg of pressed yeast (=approximately 6 kg of dry-yeast).

(III) In the third experiment, the alcoholic extract was directly fractionated with silver nitrate and baryta and proceeded in the same manner as above. The yield of purified crystals was 0.25 g from 20 kg of pressed yeast.

Properties of the hydrochloride :- The purified crystals of the hydrochloride form colorless long plates, melting at 249~250°C (uncorr.) with decomposition. Mixed with the preparation from rice-polishings, no depression was observed. Its general reactions, behaviors toward various solvents and precipitants, as well as properties were proved to be exactly identical with the hydrochloride of "oryzanin" isolated by the author from rice-polishings as shown in the following table ;

Reagents	Oryzanin hydrochloride	
	from yeast.	from rice-polishings.
Solvents :-		
Water, Alcohol, Methylalcohol, etc.	sol	sol
Acetone, Benzene, Ether, etc.	insol	insol
Precipitants :-		
Phosphotungstic acid	ppt	ppt
Mercuric chloride	ppt	ppt
Silver nitrate & baryta	ppt	ppt
Gold chloride	ppt	ppt
Platinum chloride	ppt	ppt
Picrolonic acid	ppt	ppt
Iodin-potassium-iodide	ppt	ppt
Dragendorff's reagent	ppt	ppt
Mercuric sulphate	nil	nil
Lead acetate	nil	nil
Picric acid	nil	nil
Tannic acid	nil	nil
Fravianic acid	nil	nil
Color reactions :-		
Pauly's diazo-reaction	yellow *	yellow *
Ninhydrin	nil	nil
Biuret-reaction	nil	nil
Sakaguchi's (arginin) reaction	nil	nil
Bial's pentose reaction	nil	nil
Purin reactions	nil	nil
Sulphur reaction (direct)	nil	nil
After boiling with alkali, (or fusing with metallic natrium)		
Lead acetate	black	black
Natrium nitro-prusside	violet	violet
After fusing with kalium nitrate and natrium carbonate		
Barium chloride.	white ppt	white ppt

* Differs from the proper reaction.

Analysis of the hydrochloride :-

Found No.	Subst mg	CO_2 mg	H_2O mg	C %	H %	N %	S %	Cl %
[I] Mpt. 249~250°C (uncorr)								
(1)	4.351	6.516	2.121	40.84	5.42	——	——	——
(2)	4.231	6.371	2.135	41.07	5.60	——	——	——
(3)	Dried 30 mts. at 160-170°C.							
	4.576	6.938	2.258	41.35	5.48	——	——	——
(4)	Dried 30 mts. at 160-170°C.							
	4.302	6.492	2.189	41.15	5.65	——	——	——
(5)	4.608	0.618 cc N (18°C 762 mm)			——	15.77	——	——
(6)	4.362	0.584 cc N (17°C 762 mm)			——	15.80	——	——
(7)	4.413	3.043 mg $BaSO_4$		——	——	——	9.46	——
(8)	6.153	4.086 mg $BaSO_4$		——	——	——	9.12	——
(9)	4.774	3.962 mg AgCl		——	——	——	——	20.52
(10)	4.584	3.791 mg AgCl		——	——	——	——	20.45
[II] Mpt. 249°C (uncorr)								
(11)	4.050	6.107	2.096	41.12	5.75	——	——	——
(12)	4.346	0.594 cc N (17°C 759 mm)			——	15.94	——	——
(13)	4.943	3.322 mg $BaSO_4$		——	——	——	9.23	——
(14)	4.291	3.471 mg AgCl		——	——	——	——	20.01
[III] Mpt. 249°C (uncorr)								
(15)	4.319	6.404	2.119	40.44	5.45	——	——	——
(16)	4.914	7.346	2.351	40.77	5.32	——	——	——
(17)	4.289	0.569 cc N (16°C 758 mm)			——	15.63	——	——
(18)	4.549	0.629 cc N (19°C 758 mm)			——	16.11	——	——
(19)	4.868	3.373 mg $BaSO_4$		——	——	——	9.52	——
(20)	5.945	3.966 mg $BaSO_4$		——	——	——	9.16	——
(21)	4.622	3.815 mg AgCl		——	——	——	——	20.41
(22)	4.333	3.602 mg AgCl		——	——	——	——	20.55
Calc. for $C_{12}H_{16}N_4SO_2-2HCl$				**40.79**	**5.10**	**15.86**	**9.07**	**20.11**
Calc. for $C_{12}H_{18}N_4SO_2-2HCl$				**40.56**	**5.63**	**15.78**	**9.01**	**20.00**

As there was no loss in weight by drying at 160~170°C for ½ hrs., it is very probable that the crystals have no water of crystallisation.

Picrolonate :- Light yellow needles or prisms, melting sharply at 227°C (uncorr.) with decomposition and no depression was observed when mixed with the preparation from rice-polishings. Dried at 100°C in vacuo and analysed ;

Found No.	Subst. mg	CO_2 mg	H_2O mg	C %	H %	N %	S %
(1)	4.131	7.181	1.491	47.41	4.01	- -	——
(2)	4.592	8.005	1.676	47.54	4.05	——	——
(3)	4.339	0.748 cc N (16°C 758 mm)			——	20.31	——
(4)	4.376	0.754 cc N (16°C 758 mm)			— -	20.29	——
(5)	4.245	0.738 cc N (17°C 759 mm)			——	20.43	——
(6)	8.751	2.278 mg $BaSO_4$		— -	——	——	3.69
(7)	9.435	2.676 mg $BaSO_4$		——	——	——	3 89
Calc. for $C_{12}H_{16}N_4SO_2$-$2C_{10}H_8N_4O_5$				**47.53**	**3.96**	**20.79**	**3.96**
Calc. for $C_{12}H_{18}N_4SO_2$-$2C_{10}H_8N_4O_5$				**47.48**	**4.20**	**20.74**	**3.95**
Calc. for $C_{12}H_{16}N_4SO$-$2C_{10}H_8N_4O_5$ by Windaus & his co-workers.				48.48	**4.04**	**21.21**	**4.04**

Chloraurate :- Crystallises in light orange yellow long plates melting at 189°C (uncorr.) with decomposition; soluble in alcohol and acetone, sparingly in water, but insoluble in ether and benzene etc.. Dried at 100°C in vacuo and analysed ;

Found No.	Subst mg	CO_2 mg	H_2O mg	Au mg	C %	H %	N %	S %	Cl %	Au %
(1)	4.373	2.469	0.754	1.829	15.39	1.92	- -	——	——	41.42
(2)	4.573	2.530	0.802	1.895	15.08	1.94	— -	——	-	41.44
(3)	6.229	0.312 cc N (16°C 758 mm)				——	5.83	- —	——	——
(4)	5.673	0.273 cc N (16°C 758 mm)				——	5 69	——	——	-
(5)	9.393	2.274 mg $BaSO_4$		3.903 mg Au		——	— -	3.32	— -	41.56
(6)	9.620	2.672 mg $BaSO_4$		4.013 mg Au		——	——	3.81	——	41.72
(7)	4.717	5.575 mg AgCl		1.965 mg Au		——	——	——	29.23	41.65
(8)	4.550	5.440 mg AgCl		1.889 mg Au		——	———	——	29.56	41.52
Calc. for $C_{12}H_{16}N_4SO_2$-$2HAuCl_4$					**15.00**	**1 83**	**5.83**	**3.33**	**29.58**	**41.04**
Calc. for $C_{12}H_{18}N_4SO_2$-$2HAuCl_4$					**14 97**	**2.08**	**5.82**	**3.33**	**29.52**	**40.96**

The above figures agree closely with the formula $C_{12}H_{18}N_4SO_2$ (or $C_{12}H_{18}N_4SO_2$) which was forwarded in the previous paper(1) and agreed with the formulla $C_{12}H_{20}N_4SO_2$ given by VanVeen(2) except 4-atoms of hydrogen but differed from that $C_{12}H_{16}N_4SO$ $(+H_2O)$ of Windaus and his co-workers(3) in the absence of crystal water.

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(1) S. Ohdake : Proc. Imp. Acad. Japan, **7** (1931), 102~105 ; Ibid. **8** (1932), 179~182. Bull. Agr. Chem. Soc. Japan, **8** (1932), 11~46 ; Ibid. **8** (1932), 111~121, J. Agr. Chem, Soc. Japan, **7** (1931), 775~808 ; Ibid. **9** (1933) 185~197.

(2) VanVeen : Rec. Trav. Pay.-Bas, **50** (1931), 610 ; Ibid. **51** (1932) 279 ; Zeit. Physiol. Chem. **208** (1933), 185~197.

(3) A. Windaus, R. Tschesche, H. Ruhkopf, F. Laquer, & F. Schultz : Nachrich. Göttingen, (1931), 207~213 ; Z. Physiol. Chem. **204** (1932), 123~128 ; A. Windaus, R. Tschesche, & H. Ruhkopf ; Nachrich. Göttingen, (1932), 342~346.

Activity of the hyrochloride:- The antineuritic acivity of the hydrochloride was tested on albino-rats as well as on pigeons and compared it with the hydrochloride isolated from rice-polishings.

(1) When young rats weighing about 40∼50 g were fed on the artificial diet consisting of 60% purified starch, 20% purified casein, 15% peanuts oil and 5% McCollum's salt mixture, supplemented daily with 0.4 g of autoclaved yeast and 3-drops of cod-liver oil, they developed the symptoms of B_1-deficiency usually in 4—5 weeks. By supplementing, however, with 0.001 mg of the hydrochloride per os daily they were quickly cured and grew healthy. (Chart. 1, Rat. No. 474,)

(2) Young rats fed on the same artificial diet as above, but supplemented daily with 0.001∼0.002 mg of the hydrochloride from begining of the experiment, grew normally with perfect health for 50 days. From these results, it was observed that 0.001 mg of the hydrochloride is equivalent to the international standard unit. (Chart. 1)

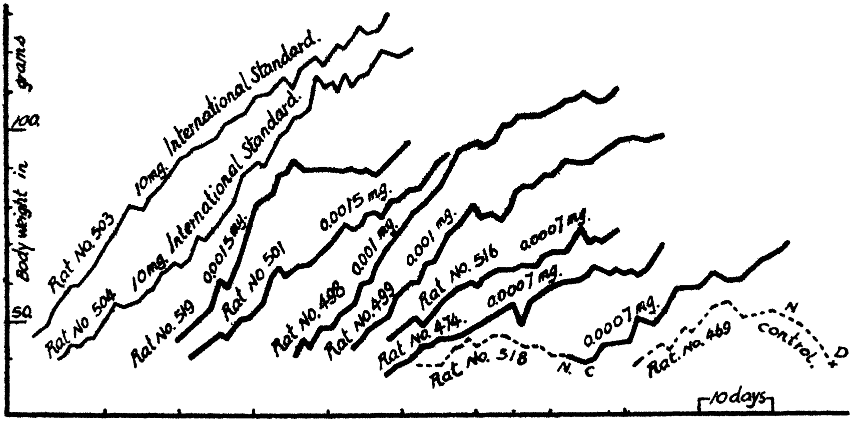
(3) Pigeons fed on the same artificial diet, developed the typical symptoms of polyneuritis after about 3-weeks, but by injecting 0.005∼0.01 mg of the hydrochloride daily, the severe symptoms were improved in 2∼3 hrs. and perfectly cured in 1∼2 days. (Chart. 2)

(4) The curative day-dose for pigeon was found to be 0.0025 mg as shown in the follwing table.

No.	Pigeon No.	Body-weight g	Days to polyneuritis.	Body-weight. suffering from polyneuritis. g	Dose injected mg	Days of protection	Day-dose mg	
(1)	455	300	27	184	0.012	6	0.0020	Average. 0.0025 mg
(2)	453	282	30	205	0 010	3	0.0033	
(3)	451	307	22	220	0.008	2	0.0040	
(4)	487	320	32	202	0.008	3	0.0027	
(5)	488	325	18	212	0 008	3	0.0027	
(6)	490(I)	295	24	224	0.008	4	0.0020	
(7)	490(II)	295	28	227	0.008	4	0.0020	
(8)	491	295	32	209	0.008	3	0 0027	
(9)	493(I)	307	16	206	0.008	5	0.0016	
(10)	493(II)	307	21	190	0.008	4	0.0020	
(11)	506	325	21	210	0.008	3	0 0027	

These results lead to the conclusion that the antineuritic substance isolated from yeast is a sulphur compound having the formula $C_{12}H_{16}N_4SO_2$ and it is identical with that isolated from rice-polishings.

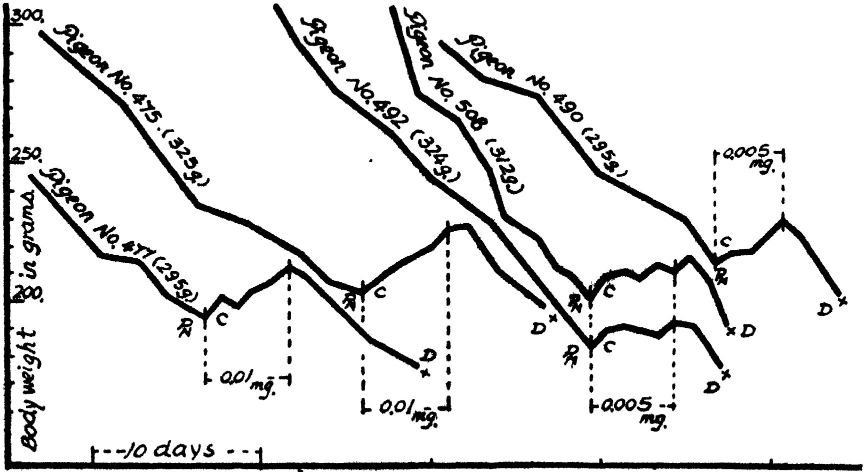
The author wishes to express his sincer thanks to Prof. U. Suzuki for his kind advise throughout the experiment and to Sankyo Company, Ltd, for kind supply of the material. Thanks are due to Messrs. M. Kamada and T. Yamagishi for their assistance both in chemical and biological experiments.



N.........B_1-Deficiency. C........ Cured. D........ Dead.

- - - - - - - - - - - - ....Artificial diet. ———————........ Artificial diet+yeast oryzanin.

Chart. 1. Albinorats on artificial diet & yeast-oryzanin.



P_N.........B_1-Deficiency. C.........Cured. D ....Dead.

Chart. 2. Pigeons on artificial diet & yeast-oryzanin.

On the Organic Bases of the Flue-Cured Tobacco.

By

Takeo Nito.

(*The Mito Experiment Station, Government Monopoly Bureau, Japan*)

(Received April 5, 1934)

This investigation has been carried out to separate the organic bases from the flue-cured tobacco.

Total nitrogen, protein nitrogen and organic base nitrogen in the sample were determined as follows:–

	% in the dry matter
Total Nitrogen	2.453
Protein Nitrogen	0.854
Organic Base Nitrogen	0.525

About 1 kg of the tobacco leaves cut finely and treated with ether, was extracted three times with hot water. From 15 litres of the above gathered extracts, the protein and other impurities were removed by neutral and basic lead acetates, and excess of the lead in the filtrate by H_2S. The filtrate was evaporated under reduced pressure to almost equal to 1/50 volume of the original extract. After the removal of Nicotin, the precipitate of the organic bases formed by phosphotungstic acid, was fractionated into the following parts.

1. A_gNO_3-precipitate (purine Fraction).

From this fraction Adenine was isolated as hydrochloride. Yield: 0.25 g. Its picrate crystallised in long yellow needles and melted at 279°C. The chloroplatinate salt of the base formed yellow column crystals and on analysing it, the following result was obtained.

Subst. taken 0.0820 g Pt 0.0237 g Pt 28.90%
Calc. for Adenine-chloroplatinate$(C_5H_5N_5HCl)_2PtCl_4$ Pt 28.70%

2. A_gNO_3 and $Ba(OH)_2$-precipitate
(Histidine and Arginine Fractions).

Histidine was clearly identified by Pauly's reaction but could not be isolated in crystalline form. And arginine was isolated as nitrate. Yield: 0.14 g. Its picrate crystallized in yellow needles and melted at 205°C. The coppernitrate salt of the base formed blue needle crystals and the analytical result is given below.

Subst. taken 0.0932 g. Cu 0.0109 g Cu 11.70%
Calc. for Arginine-coppernitrate $[(C_6H_{14}N_4O_2)_2Cu(NO_3)_2]$ Cu 11.86%

3. Fitrate from A_gNO_3 and $Ba(OH)_2$-precipitate (Lysine Fraction).

(a) Soluble part in cold absolute alcohol.

Any base was not detected.

(b) Insoluble part in cold absolute alcohol.

The picrate of the substance separated from this part crystallized in yellow columns and melted at 180°C. The chloroaurate of the base coincided with the Betaine-chloroaurate as follows :-

Subst. taken 0.0922 g Au 0.0401 g Au 43.49%
Calc. for Bataine-chloroaurate $(C_5H_{11}NO_2HCl.\ AuCl_3)$ Au 43.13%

The organic bases (except Nicotine) isolated from about 1 kg flue-cured tobacco were as follow :-

Adenine (as hydrochloride)	0.25 g
Histidine	+
Arginine (as nitrate)	0.14 g
Betaine (as chloroaurate)	0.35 g

Biochemical Studies on "Sotetsu", the Japanese Sago Plant. I.

Changes of the General Composition of "Sotetsu"-Seed during its Ripening.

By

Kotaro NISHIDA

(*Kagoshima Agricultural College, Kagoshima, Japan.*)

(Received March 6, 1934)

The "Sotetsu" (*Cycas revoluta Thunb*) is a industrial crop for starch, and being resemble to the sago plant, is called "Japanische Sagopflanzen" by German. It is a special plant of Kagoshima - and Okinawa-prefectures in our country.

Experimental Results.

I. Weight Changes of Seed during its Ripening.

The auther used for experiment the materials, which are produced on one "sotetsu" -plant growing in our college garden. The weight changes of the seed during its ripening are shown in the following table:

	30 Aug.	29 Sep.	28 Oct.	27 Nov.	27 Dec.	26 Jan.
Unhulled "sotetsu" (g)	13.25	17.30	17.75	18.00	17.65	17.36
Hulled "sotetsu" (g) (Endosperm)	5.65	9 40	10.35	10.20	10.20	10.18
Hull of "sotetsu" (g) (Spermoderm)	7.60	7.90	7.40	7.80	7.45	7.18

As is seen in the above table, the auther observed that the actual weight reaches its maximum during the ripening of "sotetsu"-seed, and then decreases in the later period.

II. Changes of Chemical Composition of seed during its Ripening.

The hulled "sotetsu" was dried and powdered; then its Chemical constituents were determined by the usual methods. The following figures are the percentage of the original weights of the hulled seeds:

	Aug. 30	Sep. 29	Oct. 28	Nov. 27	Oct. 27
Water	93.45	55.85	47.80	46.35	50.44
Dry matter	6.55	44.15	52.20	53.65	49.56
Total N	0.288	1.075	1.170	1.140	1.096
Protein N	0.145	0.942	1.075	1.103	1.084
Non-protein N	0.143	0.133	0.095	0.037	0.012
Starch	1.278	28.952	37.766	38.124	35.613
Dextrin		0 419	0.394	0.980	0.479
Reducing sugar	0.713	0.675	0.626	0.733	0.732
Non-reducing sugar	—	0.092	0.399	0.493	0.963
Crude protein	1.797	6.713	7.312	7.122	7.100
Crude fat	0.130	0.660	0.560	0.530	0.658
Crude fibre	—	1.290	0.926	0.875	0.802
Crude ash	0 476	0.880	1.081	1.238	0.981
N-free extract	4.148	34.607	42.322	43.885	40.020

III. Changes of Absolute Amount in one Seed during its Ripening.

From the above table, the absolute amount of chemical constituents in one "sotetsu"-seed was calculated as follows:

	Aug. 30	Sep. 29	Oct. 28	Nov. 27	Dec. 27
Water	5.280 g	5.250	4.947 g	4.728 g	5.145 g
Dry matter	0.370	4.150	5.403	5.472	5.055
Total N	0.016	0.101	0.121	0.116	0.112
Protein N	0.008	0.089	0.111	0.113	0.111
Non-protein N	0.008	0.012	0.010	0.003	0.001
Starch	0.072	2.721	3.909	3.889	3.633
Dextrin		0.039	0.041	0.100	0.049
Reducing sugar	0.040	0.063	0.065	0.075	0.075
Non-reducing sugar	—	0.009	0.041	0.050	0.098
Crude protein	0.102	0.631	0.757	0.726	0.724
Crude fat	0.007	0.062	0.058	0.054	0.067
Crude fibre	—	0.121	0.096	0.089	0.082
Crude ash	0.027	0.083	0.112	0.126	0.100
N-free extract	0.234	3.253	4.380	4.476	4.082

Summary.

According to the above analytical results it will be noted that:

(1) The dry matter, ash, protein, starch, dextrin and nitrogen-free extract content of the "sotetsu"-seed reaches its maximum distinctly at a definite point of the ripening period and decreases later.

(2) The sugar content of the "sotetsu"-seed increases gradually in the ripening period, on the other hand the content of fibre and non-protein nitrogen decreases gradually.

(3) In the ripening period of "sotetsu"-seed, the content of crude fat decreases gradually and increases later; but the crude protein comes up to its maximum and decreases later.

(4) The sample picked up on Aug. 30 is remarkably unriped seed, and the water content of the material is very large; then the reducing sugar, ash and non-protein nitrogen content in dried state is considerably high than that of other ripening date.

(5) From the above data, the weights of ripening endosperms and its compositions, the reasonable picking time of the "sotetsu"-seed for the raw material of starch manufacture seems to be the last decade of october to november at Kagoshima; but according to the climate even in the same district it may be more or less different.

Investigation on the Influence of Ultraviolet Rays on the Physiological Activities of Azotobacter.

I. On the lethal action of ultra-violet rays on Azotobacter chroococcum.

By

A. Itano and A. MATUURA.

(Received March 10 1934)

The lethal action of ultra-violet rays on Azotobacter chroococcum under various conditions was investigated by using Hanovia mercury lamp. First the quantity of ultra-violet rays, discharged from Hanovia lamp, was determined by both acetone methylene blue and molybdic acid methods, and found to be 3.0∼2.5 discoloration in one hour and aH 2.1∼1.6 in ten minutes respectively. The organism was exposed to the rays under various conditions, namely in Petri dish (hard glass of 29.126 transmission rate), under "Acme" ultravit-glass cover of 44.57 transmission rate and in Erlenmyer flask (hard glass of 57.888 transmission rate, measured against the quartz flask.), and obtained the following results:

Treatments	Time of exposure	Results
Petri dish	2 hrs	Not killed
"Acme" ultravit glass	1 hr.	Majority killed
	2 hrs.	Totally killed
Direct exposure	30 seconds	〃 〃
Quartz flask	5 minutes	〃 〃
	5 seconds	Stimulated the growth
Erlenmyer flask, hard glass	2 hrs.	Not killed
	30∼60 seconds	Stimulated the growth

Besides the viability of Azotobacter, the amount of nitrogen fixed and the change in the concentration of hydrogen ions under various treatments were examined. The lethal action of ultra-violet rays decreases in greater proportion than the rate of transmission of the apparatus. In all the cases, a short exposure stimulated the physiological activity of Azotobacter.

On the Effect of Linoleic Acid and Yeast upon the Growth of Rats on High Fat Diet.

By

Yoshikazu SAHASHI.

(Received January 20, 1934.)

In a previous communication⁽¹⁾ the present author reported the results of some feeding experiments on rats with synthetic diets containing large amounts of the oils obtained from sperm whale and fin-back whale.

Until recently, the chief physiological role assigned to dietary fats has been that of the fuel for the generation of energy, but certain unsaturated fatty acids are now recognized to be indispensable food factors like vitamins. The author, therefore, repeated some feeding experiments on rats using similar diets to those described in the previous paper but with the addition of linoleic acid and yeast.

Most of the rats in this experiment indicated almost normal growth, but no young were born during the entire feeding period except in the group supplied with butter. At the end of the experiment, the animals were killed and submitted to gross anatomical examination with the hope of determining the changes in sex glands. From this examination arose an unexpected discovery of the presence of vitamin E in Japanese soy bean oil, the details of which are given in a separate paper.⁽²⁾

Experimentel

(I) Samples af Fats or Oils used in the Animal Experiments.

Analysis of the samples used in the experiments:

Samples used	Sp. gr.	Refr. index	Acid value	Sap. value	Iod. value	Vitamin A Carotins
Butter (Kin en)	——	——	——	——	——	++
Beef fat***	——	——	0.6	196.7	48.2	–
Lard***	$d_4^{15} = 0.911$	$n_D^{36} = 1.471$	0.8	195.3	15.9	–
Cod liver oil (J. P. IV)	$d_4^{15} = 0.927$	$n_D^{22} = 1.475$	0.7	180.8	154.4	+++
Blubber oil* (Finback whale)	$d_4^{15} = 0.917$	$n_D^{22} = 1.466$	0.2	185.5	92.7	–
Intestine oil* (Finback whale)	$d_4^{15} = 0.916$	$n_D^{22} = 1.467$	0.9	180.6	97.6	—

These samples used in the experiments were kindly supplied by Toyo Hogei Kaisha* and Marumiya Company,** and the other ones*** were prepared from fresh raw materials in our laboratory.

Soy bean oil***	$d_4^{15} = 0.937$	$n_D^{30} = 1.481$	0.5	191.4	142.5	+
White sesame oil**	$d_4^{15} = 0.926$	$n_D^{23} = 1.471$	1.3	187.0	119.3	–
Peanut oil***	$d_4^{15} = 0.920$	$n_D^{30} = 1.475$	0.2	187.9	103.6	–
Cocoa-nut oil**	$d_4^{15} = 0.931$	$n_D^{23} = 1.452$	36.9	262.1	11.5	–
Palm oil*	——	——	——	——	——	+++
Olive oil (Betis spein)	$d_4^{15} = 0.919$	$n_D^{22} = 1.465$	0.9	191.1	92.9	–

(II) Experiment with Diets Containing a Sufficient Quantity of Fats or Oils, without the Supplementary Feeding of Linoleic Acid.

Data on the growth of rats on high fat diets without the supplementary feeding of linoleic acid will be presented first.

Albino rats weighing 40∼50 g each were previously fed for several days on a complete diet until they reached 50∼60 g. Then they were divided into several groups, each consisting of 4 rats, and were fed on various experimental synthetic diets (Charts 1 and 2). The diets consisted of:

Potato starch (Japanese Pharmacopea)	65 g	60 g
Fish protein freed from fat	18 g	15 g
McCollum's salt mixture	15 g	15 g
Oryzanin solution (Sankyo & Co.)	5 c.c.	——
Dried yeast (Oriental Co.) extracted with ether.	——	2 g
Fat or oil	15 g	20 g
Biosterin dissolved in olive oil, given per os daily	1 mg	1 mg

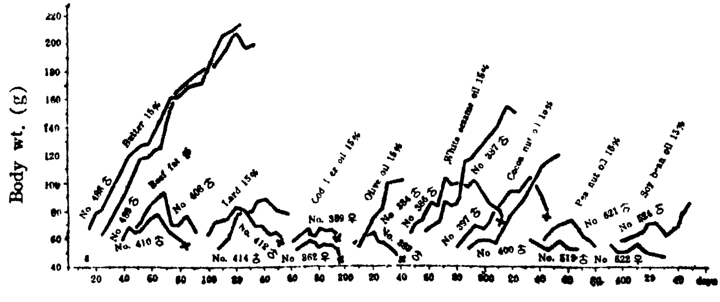


Chart 1. Growth curves of rats fed on the diets containing 15% fats or oils supplemented with oryzanin (without linoleic acid).

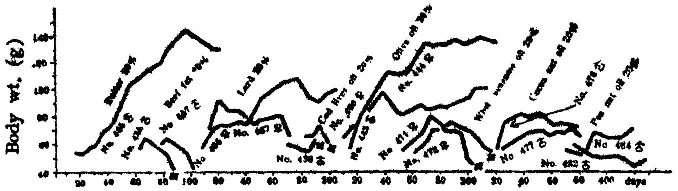


Chart 2. Growth curves of rats fed on the diets containing 20% fats or oils supplemented with dry yeast (without linoleic acid).

From the above experiment it can be seen that rats failed to grow satisfactorily on any of the diets, except on the one containing butter. Slight seborrhea was observed in some of these rats (Photo. 3).

(III) Effect of Linoleic Acid and Yeast upon the Growth of Rats fed on High Fat Diets.

We now come to the main experiment which was conducted to see the effect of linoleic acid and yeast on the growth of rats fed on high fat diets, linoleic acid being now recognized as an indispensable food factor like a vitamin.

The diets used were similar to the preceding experiment: Potato starch 65 g, fish protein 15 g, McCollum's salt mixture 4 g, dry yeast extracted with ether 2 g, fat or oil 15～20 g, and in addition biosterin 1 mg and pure

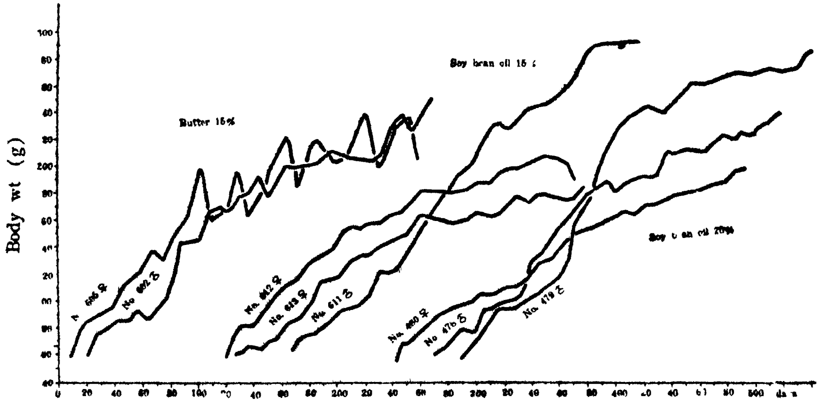


Chart 3 Growth curves of rats fed on the diets containing 15～20% butter or soy bean oil supplemented with yeast and without linoleic acid. Linoleic acid was not added since butter as well as soy bean oil naturally contains this fatty acid.

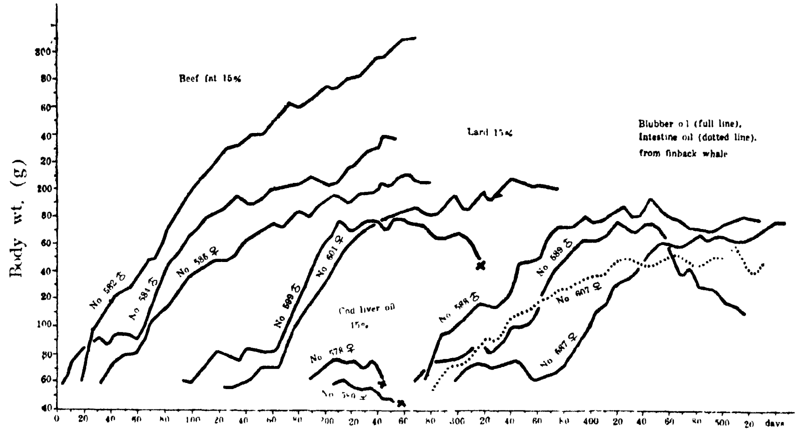


Chart 4 Growth curves of rats fed on the diets containing 15% animal fats or oils supplemented with yeast and linoleic acid,

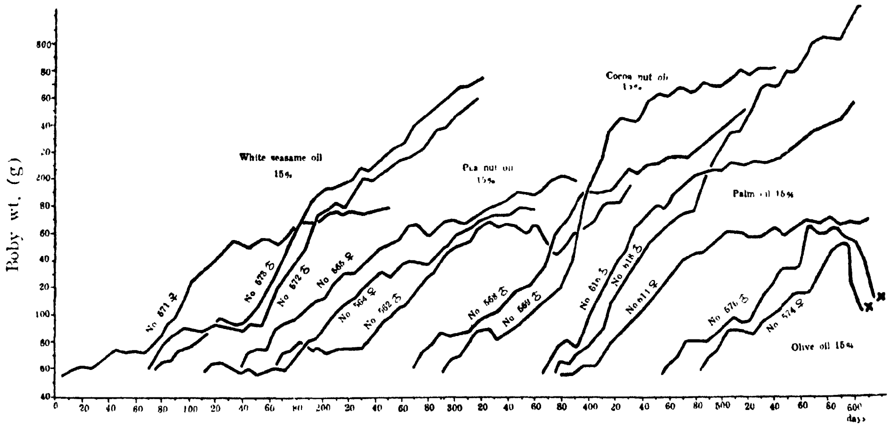


Chart 5. Growth curves of rats fed on the diets containing 15% vegetable fats or oils supplemented with yeast and linoleic acid.

linoleic acid 50~100 mg being supplied per os to every rat daily.

The rats given cod liver oil died after 50 days, but, on the contrary, those supplied with other oils and fats lived more than 250 days and secured nearly the normal growth (Charts 3~5 and Photo. 1, 2 and 4).

From the anatomical examination made on the rats at the termination of this experiment, the absence of vitamin E was recognized in all the above fats and oils except butter and soy bean oil. The evidence for the presence of vitamin E in soy bean oil is given in a separate paper.(2)

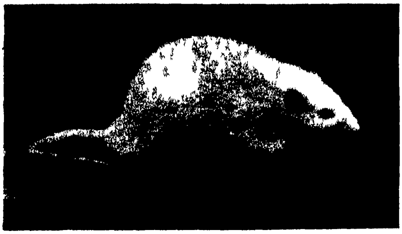


Photo 1 Rat (No 611 ♂) supplied with yeast in addition to 15% soy bean oil Body weight 288 g at the 240 th day of experiment

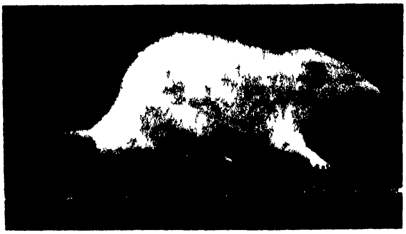


Photo 2 Rat (No 618) supplied with linoleic acid and yeast in addition to 15% palm oil Body weight 302 g at the 210 th day

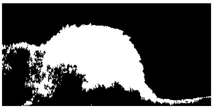


Photo 3 Slight seborrhoea in rat (No 410 ♂) fed with beef fat without yeast or linoleic acid Body weight 59 g at the 45 th day

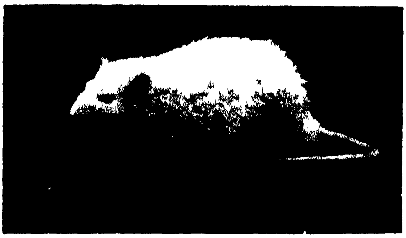


Photo 4 Rat (No 582) supplied with yeast and linoleic acid in addition to 15% beef fat Body weight 310 g at the 240 th day

Summary

Albino rats failed to secure satisfactory growth on diets containing a high percentage of any of the fats used except butter. The addition of yeast to the diet did not improve the growth of the animals. With the supplementary feeding of linoleic acid in addition to yeast the animals attained nearly the normal rate of growth, demonstrating anew the importance of linoleic acid as a dietary element

The author expresses his sincere thanks to Prof. U Suzuki for his kind advice and encouragement throughout the progress of this work

Literature

(1) Y Sahashi Sc Pap I P C R 20 245~253 (1933) Bull Agr Chem Soc Vol 9, 69 (1933)

(2) U Suzuki, W Nakahara, and Y Sahashi Sc Pap I P C R 23 No 491, 270 (1934), Bull Agr Chem Soc, Vol 10, 87 (1934)

The Occurrence of Vitamin E in Soy Bean Oil.

By

Umetaro SUZUKI, Waro NAKAHARA and Yoshikazu SAHASHI.

(Received January 20, 1934.)

Incidental to some animal experiments on the nutritional role of fats⁽¹⁾ an interesting observation was made that soy bean oil used as the only possible source of vitamin E prevented the testicular degeneration, which occurred consistently in animals receiving certain other oils. Although the general nutritive value of soy bean oil has already been investigated, the occurrence of vitamin E in this oil seems not to have been demonstrated.

In the present paper we wish to give a brief account of our preliminary experiment, which we consider sufficient to show that soy bean oil, unlike certain other vegetable oils, contains vitamin E.

Experiment

The composition of the diet used in our experiment was as follows:—

Potato starch (J. P)	65 g
Fish protein, freed of fat	15
Soy bean oil	15 or 20
McCollum's salt mixture	4
Dry yeast, extracted with ether	2

In addition biosterin, dissolved in olive oil, and pure linoleic acid were given separately to each animal in the daily dose of 1 mg and 50～100 mg respectively.

Soy bean oil was freshly expressed from soy beans in our laboratory, and it had the following properties:— Sp. gr., $d_4^{15}=0.937$; refr. index, $n_D^{30}=1.481$; acid value, 0.5; saponification value, 191.4; iodine value, 142.5. For comparison the following fats and oils were used in the same amount as and replacing soy bean oil:— Butter, blubber oil (fin-back whale), white sesame oil, peanut oil, cocoanut oil and palm oil.

Young rats were maintained on the above diet, with one of the oils as the source of fat. The rats grew nearly at the normal rate during the experimental period of 250 days, many attaining the body weight of 250～300 g. For the growth curves of these rats the reader is referred to a separate paper by one of us (Sahashi).

At the end of the experimental period of 250 days, there were 13 male rats distributed to different oil groups, and all these were killed and autopsy *performed.*

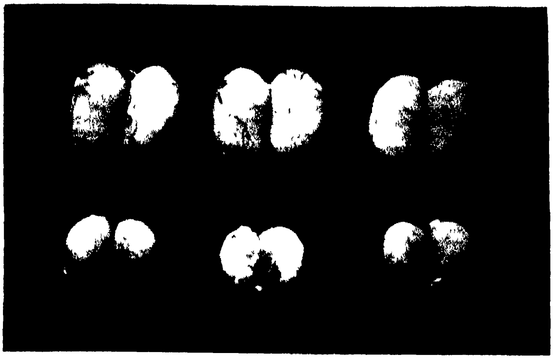
No important pathological change was noted in any of the rats. Slight and localised inflammation in the lung, and a small number of tapeworm cysts in the liver were encountered in a few cases, but these findings are of no significance. We found, however, that testicles were in the state of very marked degeneration in many of the rats, as may be seen from their weights given in the following table:—

Table I.

Oils	Rat No.	Weight of testicle
Soy bean oil	478 479 611	1.10 g each 1.20 g each 1.20 g each
Peanut oil	562	0.40 g 0.50 g
Cocoanut oil	568	0.40 g each
	569	0.40 g 0.35 g
White sesame oil	572	0.55 g 0.60 g
	573	0.55 g 0.65 g
Palm oil	616 618	0.40 g each 0.70 g each
Blubber oil	588 589	0.35 g each 0.35 g each
Butter	602	1.10 g each

The degenerated testicles were not only small in size but were also very soft, not associated with signs of inflammation. In the accompanying photograph are shown three pairs of such degenerated testicles (lower row) in comparison with three other pairs (upper row) of perfectly normal testicles taken from the three soy bean oil rats. It is highly significant that testicles of the soy bean oil fed rats were absolutely normal in every way. Butter fed animal also showed normal testicles, but all the rest of the rats were suffering from marked testicular degeneration.

That so marked a degeneration of testicles should occur in spite of the generally excellent physical conditions of the rats is significant, and a consideration of the composition of the diet leaves little doubt that here we



Upper row of three pairs of normal testicles are from the rats fed with soy bean oil. In the lower row are three pairs of degenerated testicles from rats of cocoanut oil and peanut oil groups. The more or less uneven surface of the degenerated testicle is due to the shrinkage characteristic of such testicles preserved in 10% formalin. Approximately natural size.

are dealing with the case of vitamin E deficiency. The large testicles, normal in every way, of the soy bean oil rats, then, constitute evidence that this oil contains vitamin E.

Additional experiments are now being conducted with the object of determining the effect of soy bean oil on the reproduction of rats. These experiments have not as yet progressed far enough to be published, but we have already obtained sufficient evidence to show that reproduction will take place on a synthetic diet with soy bean oil as the only possible source of vitamin E. We hope to give the details of these experiments in our next publication.

Conclusion

The incorporation of a liberal amount of soy bean oil to otherwise vitamin E deficient synthetic diet prevents the development of the testicular degeneration characteristic of vitamin E deficiency. This fact, taken together with certain evidence of fertility of rats on the same diet (unfinished experiments) leads us to conclude that soy bean oil contains vitamin E.

Literature.

(1) Y. Sahashi: Sc. Pap. I. P. C. R., **23**, No. 490, 264, (1934); Bull. Agr. Chem. Soc., Vol. **10**, 62 (1934).

Über die Verteilung der Manganverbindung im Boden Japans, und ihre Beziehung auf die Fruchtbarkeit.

Von

Chikafumi ICHIKAWA.

(Eingegangen am 19 Februar, 1934)

Mit 58 Proben des Bodens, die aus verschiedenen Provinzen in Japan gebracht worden waren, habe ich über die Enthaltung der Manganverbindung studiert.

Dabei ist sie in all diesen Proben betrachtet warden;

1. Der Enthaltungsgrad der von HCl gelösten Manganverbindung ist von Spur zu höchstens 0,612%, und desselbe der von KCl- lösung gelösten ist auch von Spur zu höchstens 30,67 mg in 100 g Probe.
2. Die von HCl oder KCl gelösten Manganverbindungen sehen im Boden aus jeder Provinz in Japan zu existieren aus.

Und der Enthaltungsgrad derselben hat ein Verhaltnis zu die Fruchtbarkeit des Bodens.

3. Hierbei scheint es mir, je grosser der Vergleichsgrad zwischen die Quantität der von HCl gelosten Manganverbindung und dieselbe der von KCl gelösten ist, um so Fruchtbarer ist der Boden.

Researches on the Electric Boundary Layer Disturbance. Part IV

The Effect of the Alternating Electric Field on the Permeability Potential of Membrane

By

Masuzo SHIKATA and Koichiro KITAO

(*The Chemical Laboratory of Forest Products, Kyoto Imperial University*)

(Received May 25, 1934)

Prof. Michaelis has shown that the potential difference was always observed between each side of collodion membrane when the KCl solutions of different concentration was separated by this membrane. ,

He ascribed this phenomenon to the difference of mobilities of K ion and Cl' ion through this membrane.

The authors have observed that if this membrane was once under the influence of an alternating electric field, for example during 10 to 30 seconds in the 220 A. C. field of 60 cycles, the potential difference was almost disappeared, and after 5 to 10 minutes it returned to the initial value.

Chemical Researches on the Pulp Woods in Karafuto. Part V

Cooking and Spinning Tests of Karafuto Fir

By

Masuzo SHIKATA and Takeo KOHSAKA

(The Chemical Laboratory of Forest Products, Kyoto Imperial University)

(Received May 25, 1934)

The result of chemical analysis of the Akatodomatsu (*Abies sachliensis Fr. Schem.*) of Karafuto was given in table (I). No remarkable difference has been observed between three kinds of Akatodomatsu (i. e. three kinds; fir with long, medium and short bract-scales).

Although the total cellulose content of Karafuto fir is nearly the same as Karafuto spruce, Ezomatsu, (*Picea ajanensis Fisch.*), the alpha-cellulose content of the former is conspicuously less than that of the latter. We may conclude that the fir is much inferior to the spruce as the rayon pulp wood.

The result of cooking test is given in table (2). Ammonium bisulphite methods give better yields than magnesium bisulphite methods. The viscose silks have been prepared from these pulps and the Canadian Kipawa pulp. The tensile strength of these viscose silks have been tested.

Table I

The result of chemical analysis of the Akatodomatsu.

	fir with long bract-scales absolute dry%	fir with medium b. s. absolute dry%	fir with short bract-scales absolute dry%
Alcohol-benzene ext. substance	3.63	3.86	2.97
1%NaOH soluble matter	13.51	14.55	12.95

Hot water solu. matter	4.49	5.19	3.89
Cold water solu. matter	2.33	2.30	2.60
Total cellulose	55.35	55.47	55.23
Alpha-cellulose	34.82	34.84	33.89
Beta and gamma-cellulose	20.53	20.63	21.34
Lignin	30.21	30.42	29.07
Pentosan	10.99	11.60	13.68
Mannan	5.31	4.90	3.94
Galactan	0.25	0.25	0.23
Hemicellulose (10, 11, 12)	16.47	16.75	17.84
Methoxyl-radical	5.17	5.24	4.94
Nitrogen	0.08	0.08	0.07
Crude protein	0.51	0.48	0.46
Ash	0.41	0.42	0.40
Methoxyl-lignin ratio (%)	(17.12)	(17.20)	(17.00)

Table 2

Cooking and Spinning test

cooking solution	Mg-bisulphite	NH_4-bisulphite
Total SO_2	6.2%	6.2%
Base	MgO 1%	NH_3 1%
Maximum pressure	7 atm. p.	5.5 atm. p.
Mean pressure	4.5 atm. p.	4 atm. p.
Maximum temperature	155°C	145°C
Mean temperature	130°C	125~130°C
Time	10 hrs.	10 hrs.
Yield	35~36%	39~40%
Alpha-cellulose content in pulp	80%	75~80%
Tensile strength of the viscose silk	0.7 g/dn.	0.7 g/dn.

On the Production of Seborrhea in Rat by Feeding with whale Oil. Part I.*

By

Eiichi SOMEKAWA.

(Received April 20, 1934.)

In the previous experiments, carried out by the author in cooperation with Dr. W. Nakahara, on the effect of various fats and oils upon the growth of rat tumor, it was observed that the rat when fed on a diet containing 10~15% whale oil (on sale containing about 40% unsaponifiable matter) develops seborrhea, characterized by the secretion of oily substance from the skin, the hair becoming completely imbibed with the oil so that the animal looks as if it were dipped in oil. The body weight gradually decreased and the animal finally succumbed within 2~3 weeks. The same disease is also produced by common sperm oil or arctic sperm oil.

As such a disease produced by whale oil is not yet recorded anywhere, the author has studied it more closely and tried to isolate the active substance which causes this curious disease. It is well known that sperm oil consists of mixed waxes, i. e. the mixed ester of cetyl alcohol, oleyl alcohol, etc. with fatty acids, such as palmitic, myristic, oleic etc. The solid wax which deposits on cooling the whale oil is cetyl palmitate, and the greater part of the liquid wax is presumed to consist of oleic acid ester of oleyl alcohol, i. e. oleyl oleate.

The author has observed that the characteristic sympton is no more produced when whale oil is saponified though the toxic property is increased. The author thus suspected that the liquid wax contained in whale oil might be the active substance. From such a point of view the author has prepared the oleic acid ester of oleyl alcohol synthetically and confirmed that exactly the same symptom is produced with this substance as with whale oil.

Y. Sahashi(1) in our laboratory has studied the same subject and observed that the solid wax (cetin, cetyl palmitate) has no toxicity because it is not assimilated at all, while oleyl alcohol has a very noxious action without producing seborrhea. It is in accordance with the author's view that the said disease is produced by the liquid wax (oleyl oleate) which on saponification loses the property of producing the disease though the saponified products act more noxiously.

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* This paper was published in Sc. Pap. I. P. C. R., 21 (1933), 149—157.

Experimental

The blubber oil prepared from the bottlenose whale by Tōkai Fishery Company in Tateyama, Awa province, was used for the following experiment. It has the following properties:

Acid value	0.7
Iodine value	83.9
Saponification value	116.6
Unsaponifiable matter (%)	42.1

The composition of the diet was as follows:

Fish protein	15%
Starch	65%
Whale oil	15%
McCollum salt	5%
Oryzanin	5 cc
Riken-Vitamin A	1 drop per rat per day

When young male rats weighing 50～60 g were fed on the above diet, they could not grow at all, an oily substance diffused out over the skin, first around the anus and gradually extended over the whole body and at last the animals presented such an appearance as if they were dipped in oil (Table I, Fig. 1).

Table I.

Rat No.	Body weight (g)		Gain or loss in body weight (g)
	Initial	At 7 th day	
157	59	57 (Death)	− 2
158	56	54 (〃)	− 2
159	58	47 (〃)	−11
160	58	62	+ 4

In the next experiment, instead of oryzanin, 5 g of dry yeast were added per 100 g of diet. In this case the rat survived somewhat longer but suffered from the same disease as bofore (Table II. Fig. 2).

Table II.

Rat No.	Body weight (g)		Gain or loss in body weight (g)
	Ioitial	At 7 th day	
161	58	46	−12(Death at 9th day)
162	55	59	+ 4(〃 〃 〃 〃)
163	56	60	+ 4(〃 〃 10th 〃)
164	56	66	+10(〃 〃 11th 〃)

In the third experiment, 10 resp. 15% of dry yeast were added to the diet as follows:

	(A)	(B)
Fish protein	15%	15%
Starch	55%	50%
Whale oil	15%	15%
Dry yeast	10%	15%
McCollum salt	5%	5%
Riken-Vitamin A	1 drop per rat per day	

After feeding 1∼2 weeks on the above diets, the skin symptom appeared in both groups similarly. In group (A) no distinct symptom was observed for 5∼8 drys, the growth was better than in group (B), but afterwards the disease developed rapidly and after 2 weeks it became quite the same as in group (B) (Table III, Fig. 3 and 4).

Table III.

	Rat No	Body weight (g)		Gain or loss in body weight (g)
		Initial	At 14th day	
(A)	187	55	(Death at 8th day)	
	188	61	58	− 3
	189	60	65	+ 5
	190	60	68	+ 8
(B)	193	53	71 (At 7th day)	+18
	194	49	(Death at 6th day)	
	195	51	(〃 〃 5th 〃)	
	196	49	(〃 〃 7th 〃)	

We see from the above results that the noxious action of whale oil cannot be prevented even by adding 15% yeast to the diet.

On the whole, the symptom seemed to run parallel with the growth of rat; that is to say, the rat which grew relatively well, with large food intake presented more remarkable symptom.

Experiments with the unsaponifiable matter and fatty acids of whale oil.

(A) Saponification of whale oil: The oil was saponified by boiling with three times of its weight of 10% alcoholic potash for 2 hours, hereupon about one third of alcohol was distilled off, and after diluting with about five times of water the unsaponifiable matter was repeatedly extracted with ether until the ethereal soulution became colourless. The combined ether extract was then distilled and the residue thus obtained was saponified once more with a little alcoholic potash by warming for a short time. After diluting with water the unsaponifiable matter was extracted with ether as before, dehydrated with anhydrous sodium sulphate and the ether was distilled off.

The soap solution, from which the unsaponifiable matter had been removed, was acidified with dilute hydrochloric acid and the liberated free fatty acids were extracted with ether, washed with water, dehydrated and the ether was distilled off as before. W. Fahrion⁽²⁾ has pointed out the presence of cetyl alcohol ($C_{16}H_{34}O$) in arctic sperm oil, and M. Tsujimoto⁽³⁾ has shown that the unsaponifiable matters of sperm head oil and arctic sperm oil contain cetyl and oleyl alcohol ($C_{18}H_{36}O$).

(B) Separation of unsapnifiable matter: The separation of unsaponifiable matter into solid and liquid portions was effected by the method of Tsujimoto⁽⁴⁾ as follows:—The unsaponifiable matter was dissolved in three times of its weight of acetone, cooled with freezing mixture for 1 hour and the separated crystalline substance was collected on a Buchner funnel and washed with a small amount of cold acetone. From the filtrate, after distilling off the acetone and cooling, a second crop of crystals was obtained. It was united with the first crystals and recrystallized twice from 80% alcohol or acetone; m p 49~50°.

The liquid portion was distilled at 13 mm, b p 170~208°; iodine value 85.5.

(C) Feeding experimets were now carried out with the unsaponifiable matter and fatty acids prepared as described above in order to determine which of the two is the active substance.

Composition of diets:

(A)	
Fish protein	15%
Starch	75%
Fatty acids	5%
McCollum salt	5%
Oryzanin	5 c c.
Riken-Vitamin A	1 drop per rat per day

(B)	
Fish protein	15%
Starch	68%
Butter	10%
McCollum salt	5%
Solid unsap. matter	2%
Oryzanin	5 cc

(C)	
Fish protein	15%
Starch	68%
Butter	10%
McCollum salt	5%
Liquid unsap. matter	2%
Oryzanin	5 cc

	(D)	(E)
Fish protein	15%	15%
Starch	70%	65%
Unsap. mattr + Fatty acids	10%	15%
McCollum salt	5%	5%
Oryzanin	5 cc	5 cc
Riken-Vitamin A	1 drop per rat per day	

The fatty acids, as well as the solid and liquid unsaponifiable matters added to the above diets (A), (B) and (C), were prepared from sperm head oil, while those prepared from arctic sperm oil, were used for (D) and (E).

Both of the oils contained about 40% unsaponifiable matter and 60%

fatty acids, and the unsaponifiable matter of sperm head oil contained roughly the same amount of solid and liquid portions, so that the contents of fatty acids and unsaponifiable matter in the diets (A), (B) and (C), were approximately equivalent to 10% of the original oil. This amount seems to be the minimum for producing the characteristic symptom on rat.

The sample used for group (D) and (E), was the mixture of 40% unsaponifiable matter and 60% fatty acids. The results of feeding experiments were as follows (Table IV):

Table IV.

	Rat No.	Body Weight (g)		Gain or loss in body weight (g)
		Initial	At 4 weeks	
(A)	73	47	73	+26
	74	52	(Death at 4th day)	
	75	51	(〃 〃 14th 〃)	
	76	48	(〃 〃 15th 〃)	
(B)	77	52	(Death at 15th day)	
	78	46	62	+16
	79	47	77	+30
	80	52	50	− 2
(C)	81	49	46	− 3
	82	51	(Death at 16th day)	
	83	48	61	+13
	84	47	66	+19
(D)	131	55	(Days survived) 10	
	132	56	14	
	133	52	12	
	134	52	4	
(E)	169	57	2	
	170	58	5	
	171	53	2	
	172	53	3	

It the above experiment, no characteristic symptom was developed in any groups, so it seems that this property is lost by saponification. However, the saponification products (fatty acids and wax alcohol) exerted more harmful effect than the original oil.

The experiment with the fatty acid ester of wax alcohol.

As described above, the saponification product, namely, fatty acids as well as the unsaponifiable matter, produced no characteristic symptom on rats. However, the author suspected that the fatty acid ester of wax alcohol may exert the same action as whale oil and prepared synthetically the wax ester. Y. Toyama⁽⁵⁾ has pointed out that the fatty acid of arctic sperm oil is largely the unsaturated acid of oleic acid series, so the author prepared the oleic acid ester of oleyl alcohol. Oleyl alcohol was prepared from the liquid fraction of the unsaponifiable matter by distilling two or three times fractionally under reduced pressure, and the fraction distilling at 8 mm, 193 ~200° was collected. The yield was more than 50% of the unsaponifiable matter; iodine value 90.46~92.21 (calc. 94.7). Its purity was further ascertained by elemental analysis (analyzed by Mr. Iki):

	Subst. (mg)	Co_2 (mg)	H_2O (mg)	C (%)	H (%)
found	4.470	13.140	5.518	80.17	13.81
calculated for $C_{18}H_{36}O$				80.94	13.10

Oleic acid used was Merck's preparation (extra pure). It was once more distilled under reduced pressure before using.

(A) Preparation of oleyl oleate: Oleic acid was converted into the acid chloride by treating with phosphorus trichloride. Twentyfive grams of oleyl alcohol were dissolved in a mixture of 50 cc of anhydrous benzol and 30 cc of anhydrous pyridine, and a slight excess of the chloride of oleic acid dissolved in 50 cc of anhydrous benzol, was added with cooling, allowed to stand at room temperature overnight, and after heating for 4 hours on the water-bath, benzol was distilled off under reduced pressure and the resulting reaction product was dissolved in petroleum ether (40~60°), washed with dilute sulfuric acid, made alkaline with 40~50% alcoholic potash and petroleum ether was renewed two or three times. The combined petroleum ether extract was washed one or two times with the above potassium hydroxide and then repeatedly with water, in order to remove the soap and alkali completely, and after dehydrating with anhydrous sodium sulfate, the solvent was distilled off in CO_2-atmosphere. The wax ester thus obtained, still contained some free oleyl alcohol and acid, so it was repeatedly washed with absolute alcohol, and a little alcohol adhered to the ester was expelled off by warming in vacuum. An almost colourless oily liquid was thus obtained; yield 40 g (ca. 80% of the theory); saponification value 105.65 (calc. 105.25). This sample was analyzed by Mr. Iki with the following result:

	Subst. (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
found	3.391	10.01	3.98	80.51	13.13
calculated for $C_{18}H_{35}O\ CO{\cdot}C_{17}H_{33}$				81.20	12.78

(B) Feeding experiments were carried out with the synthetic oleyl oleate prepared as described above:

Composition of diet:

Fish protein	15%
Starch	65%
Oleyl oleate	**15%**
McCollum salt	5%
Oryzanin	5 cc
Riken-Vitamin A	1 drop per rat per day

By feeding on the above diet, an oily substance diffused out over the skin around the anus of rat after 1∼2 days, and finally the animals developed exactly the same symptom as in the case of whale oil. The similar experiment was repeated with the crude ester prepared by different methods with the same result. Oleyl palmitate and cetyl oleate, prepared by the above-mentioned method, did not show any distinct effect on rat, a slight symptom appearing at the beginning disappeared quickly (Table V, Fig. 5).

Table V.

Rat No.	Body weight (g)		Gain or loss in body weight (g)
	Initial	At 10th day	
203	59	55	− 4
204	58	51	− 7
205	62	59 (7th day)	− 3

From the above results it was proved that the synthetic oleyl oleate has the same action as whale oil.

Summary

1. When rats were fed on sperm head oil as well as on arctic sperm oil (oils of skin, muscle and bone), adding 10∼15% in the diet, an oily substance diffused out from the skin and the animals show such an appearance as if they were dipped in oil. Besides, the above oils are very noxious, retarding the growth and finally causing the death of animals in a few weeks.

2. The above symptom produced by the whale oil cannot be prevented by adding 15% yeast in the diet, though the toxicity seems to be somewhat reduced, but rather the addition of yeast favor the development of more conspicuous symptom.

3. Saponification products of whale oil, namely, fatty acids and unsaponifiable matter, produced no such symptom as the original oils, but the toxicity was stronger than the latter. Moreover, it is not decided whether or not the substance which causes seborrhea is identical with that which prevents the growth of the animal.

4. The severe symptom appeared on these animals which grew relatively well with good food intake.

5. The oleic acid ester of oleyl alcohol synthetically prepared by the author exerted the same effect as whale oil, but oleyl palmitate or cetyl oleate gave no such phenomena.

In conclusion, the author wishes to express his sincere thanks to Prof. U. Suzuki for his kind guidance. Thanks are also due to the Tōkai Fishery Company and to Mr. K. Tashima for their kindness in supplying the materials.

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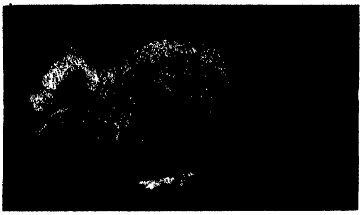

Fig. 1—Blubber oil of bottlenose whale 15%, 7th day. No. 160.

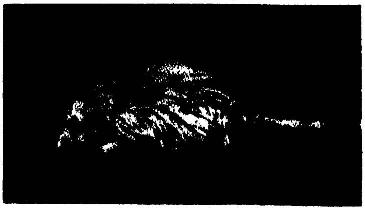

Fig. 2—Yeast 5 g per 100 g of diet (whale oil 15%), 8th day. 161.

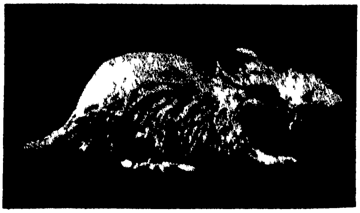

Fig. 3—Yeast 10% (whale oil 15%), 13th day. No. 190.

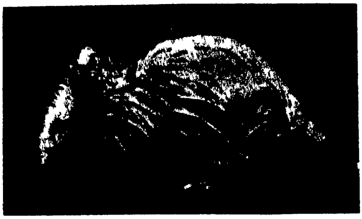

Fig. 4—Yeast 15% (whale oil 15%), 7th day. No. 193.

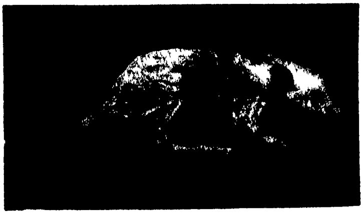

Fig. 5—Synthetic oleyl oleate 15%, 10th day. No. 203

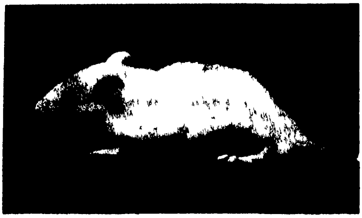

Fig. 6—Normal rat.

Investigation on the Influence of Ultra-violet Rays on the Physiological Activities of Azotobacter. II.

On the Stimulation of Azotobacter Chroococcum by Ultra-violet Rays.

By

Arao ITANO and Akira MATUURA.

(Received June 19, 1934.)

The preceding paper (Jour. Agr. Chem. Soc., Japan. X: 477～485, 1934.) dealt with the lethal action of ultra-violet rays on Azotobacter chroococcum, and in the course of investigation, it was found that a short exposure stimulated the physiological activity of Azotobacter, and in this investigation, the following points were investigated and the results are reported: 1. Rate of stimulation by different length of exposure, determined by the number of cells and also by the change of pH in the medium; 2. Mechanism of stimulation, the influence of ultra-violet rays on the physical properties of medium and their subsequent influence on Azotobacter; 3. Manner of exposure, continuous or intermittent.

The method used is the same as described previously, using Hanovia mercury lamp as the source of ultra-violet rays and exposed Azotobacter chroococcum for different intervals in Ashby's solution medium placed in Erlenmyer flask of hard glass, and obtained the following results: 1. The greatest number of cells was obtained by one minute exposure, and the longer exposure caused the decrease of cells. The change of pH in medium was greatest on one minute exposure and the reaction became acid; 2. It was ascertained that the increase of cells was brought about chiefly by the action of ultra-violet rays such other factors as the heat rays, visible rays and the change of physical properties of medium have little influence; 3. The influence of ultra-violet rays on the physical properties of medium such as electrical conductance, pH, osmotic pressure, viscosity and surface tension, was determined and found that the change of electrical conductance, pH and osmotic pressure was comparatively greater than that of other properties, and the reducing action was noted, although it is not sufficient to consider the reduction as an important factor which brings about the stimulation; 4. So far as the manner of exposure is concerned, the better stimulation was obtained by the continuous exposure than by the intermittent within one minute, but when the exposure was longer, the intermittent exposure was better owing to the lethal action of continuous exposure.

Studies on the Digestion of the Carbohydrates Contained in Mulberry Leaves by Silkworms. Part II.

On the Digestibility and the Quantity of the Digestion of the Carbohydrates Contained in Mulberry Leaves in Different Stages of Growth.

By

Kiyotoki KATO.

(At the Sericultural Experimental Institute of Kumamoto Prefecture.)

(Received June 23, 1934)

E. Hiratsuka had been studied on the digestion of the carbohydrates contained in mulberry leaves by silkworms already. Writer also have been studied on this problem and reported in the first paper. On this problem there are many important matters to be studing on the standpoint of solving the problems of mulberry leaves nature. Now, writer would to be studing on these problems continuously. This report is the experimental results on the digestibility of the carbohydrates contained in mulberry leaves in different stages of growth.

Results are as follows.

(1) The digestibility and the digested quantity of the carbohydrates (total soluble carbohydrate, reducing sugar and canesugar in each) contained in mulberry leaves by silkworms vary with the stages of growth of mulberry leaves, namely, in younger and older leaves are small, middle leaves is larger. The digestibility of the carbohydrates is found to be correlated to the quantity of the corbohydrates contained in the leaves.

(2) The accumulated quantity of the fat and glycogen in silkworms and silkworm-chrysalis varies with the different stages of leaves, and this relation is similar to the relation of the digestibility of carbohydrates with the growth of leaves.

Chemical Studies on "Bukuryo", Sclerotia of *Pachyma Hoelen* RUMPH. (IV).

Discussion of the Relation Among β-*Pacyman* and Related Compounds.

By

Kenzi TAKEDA.

(*Agricultural Chemical Laboratory, Tottori Agricultural College, Tottori.*)

(Received June 28, 1934.)

A study was made of the physical and the chemical properties of β-pachyman isolated from "Bukuryo", from the standpoint of the comparison with those of the closely related compounds, some of which have been considered by certain investigators, as identical, and the following conclusion has been made.

The author's β-pachyman is confirmed to be an independent carbohydrate, different from fongose (Tanret), callose (Mangin) or paradextran (Winterstein), and this should be likely, on the contrary, accepted as the same, in essence, as pectose (Gore), pachymose (Champion and Winterstein) and a carbohydrate which Wang and Takao isolated from "Bukuryo" in the different method from the case of the author's β-pachyman.

Moreover, it was found to be true that Tanret's fongose, at least, consists of two kinds of sugars, fongose I and II.

Chemical Studies on "Bukuryo", Sclerotia of *Pachyma Hoelen* RUMPH. (V).

Nutritive Value of *Pachyman.*

By

Kenzi TAKEDA.

(Received June 28, 1934.)

Albino rats were used and the digestion coefficient was studied with three kinds of "Bukuryo" preparations which were obtained by various methods of heating. The first was heated at 40° in water for 1.5 hours and

this treatment was repeated four times (A). In the second case, the temperature was the only factor which was varied, the water being brought to the boiling point (B). The third was autoclaved at 120° in double atmospheric pressure for 1.5 hours (C). These three samples were respectively filtered, washed throughly with water, dried and used for the feeding experiments. The composition of the rations used were as follows:

Ration I.	
"Bukuryo" preparation	14.0
Casein (Merck)	14.0
Fat free dried beef powder	4.0
McCollum's salt	3.0
Cod liver oil	3.0
Oryzanin	2.0

Ration II.	
Ration I (C)	50.0
Dried white bread	50.0

From two to five rats were used for each ration and the observation was made for each individual rat with the following conclusions.

(1) No remarkable difference in digestion coefficient could be found among these three kinds of pachyman, through the use of treated "Bukuryo", though the gradual increase of the digestion coefficient was recognized, as the temperature rose.

(2) When the content of pachyman in the ration (dry matter) was 60 ~66% and the nutritive ratio was narrow, ranging from 3.5 to 3.6 and no soluble sugar was contained, the maximum digestion coefficient of pachyman was in all cases lower than 55%.

(3) When the ration II which contained appreciable amount of digestible carbohydrate was used, the digestion coefficient of pachyman became extremely low, i. e. 18%.

(4) The nutritive value of pachyman resembled somewhat that of cellulose, and therefore, it can not be used in place of other soluble polysaccharides such as dextrin, starch or glycogen.

(5) Poor nutritive value of pachyman, which is the principal constituent of "Bukuryo" and the small amount of effective nutrients, make the nutritive value of "Bukuryo" itself less valuable, from the standpoint of nutrition, and it is highly probable that this can not be used as substitute for cereal grains.

On the Non-identity of Adenine and Vitamin B_4.

By

C. Y. Chen.

(*From the agricultural Chemical Laboratory, Tokyo Imperial University, Komaba, Tokyo.*)

(Received June 8, 1934)

In a recent publication R. Tscheshe⁽¹⁾ has directed attention to the similarity between adenine hydrochloride and the crystals isolated by Barnes and Reader⁽²⁾ which were stated by them to have vitamin B_4 potency (10 r per diem per rat). Later, Heard, Kinersley, Peter and Reader⁽³⁾ showed that B_4 is not identical with adenine, Reader tested a specimen of adenine hydrochloride for B_4 activity with negative results. Neither did irradiated adenine produce a vitamin B_4 response.

The present author has also carried out some feeding experiments with albino-rats and come to the conclusion that adenine has no B_4 potency. The details are given in the following.

Experimental.

The adenine preparation used in this experiment was kindly furnished by Dr. S. Ohdake in this laboratory. It was isolated from dried brewers yeast and was proved to be quite pure. It melted at 360～365° and gave the characteristic purple red coloration by gently heating with zinc and hydrochloric acid on a water bath.

The experimental procedure was essentially the same with that outlined by Reader⁽¹⁾. Young albino rats weighing 50～55 g were kept, each separately, in metal cages provided with wire-screen false bottoms. The Vitamin B_4 free basal diet was also prepared according to the direction of Reader⁽⁴⁾ with certain modification. It was composed of purified casein, 20% ; purified starch, 75% ; McCollum's salt mixture, 3% ; and cod liver oil, 2%.

The casein was prepared from commercial product by dissolving in dilute ammonium hydroxide and precipitating with dilute acetic acid according to a modification of the method of Bosworth and Van Slyke⁽⁸⁾. The precipitated casein was then held for 5 days in 0.01% acetic acid, the acid being changed at least once a day. Next the casein was treated with 95% ethyl alcohol, and finally extracted with ether. The resultant white, friable, tasteless product was dried in a current of warm air and ground to a fine powder.

The starch was extracted for three days with 95% ethyl alcohol and then with ether to remove water soluble vitamins. It has been shown to be pure enough for this purpose. The cod liver oil was incorporated in the basal diet which was stored in a refrigerator for daily use.

Besides, the Vitamin B_2 used in the present investigation was prepared by a method described by T. Naraywan and Drummond(6) and 15 mg per day per rat were found to be sufficient. Further, oryzanin concentrate prepared by Dr. Ohdake was used as the source of B_1. This preparation was highly active and quite free from B_2 or B_4. One rat unit or approx. 0.002 mg being given to each rat per os, daily. The experimental animals were divided into two series, i. e. the curative and preventive. In the former, the rats were first fed on a basal diet alone until the typical symptoms of B_4 deficiency were developed, i. e. the decrease of body weight, with spastic gait, a tendency to sit in a hunched position etc. as described by Reader(4)(5). At that time adenine was administered at the rate of 20 mg per rat per day. In the latter, the animals were fed on the same basal diet supplementing adenine from the beginning of the experiment. For this purpose a definite amount of adenine was mixed with a small quantity of the basal diet and given to each rat. The rats were very soon found to consume this ration without objection.

Results.

The results of the experiments are shown in Figs. I, II, III and IV. From these observations, it must be concluded that adenine is ineffective in

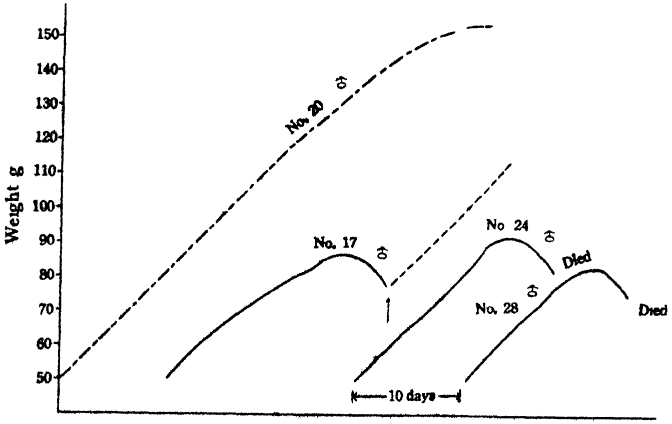

Fig I. Shows the growth curves of rats fed on a diet in which Adenine was the sole source of Vitamin B_4

———— Growth curves of the rat fed on a basal diet supplemented with Adenine

-------- Adenine was replaced with 0.4 g of dry yeast daily

—-—-— Basal diet supplemented with 5% dry yeast.

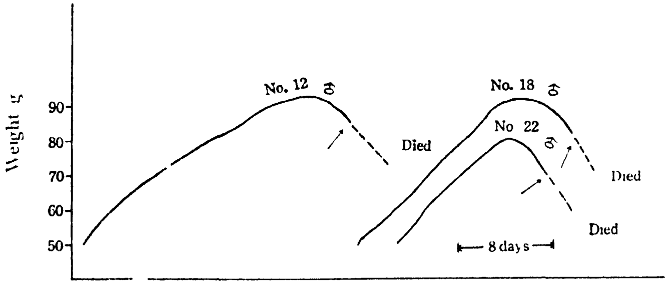

Fig II Shows the growth curves of rats fed on a P_1 free diet, Adenine or Adenyl thio methyl pentose were supplied as the source of P_1 at the point marked by the arrow in each group

——————— Growth curves on a basal diet alone
ditto supplemented with 20 mg Adenine daily
- - - - - - - - ditto supplemented with 0 05 g Adenyl thio methyl pentose

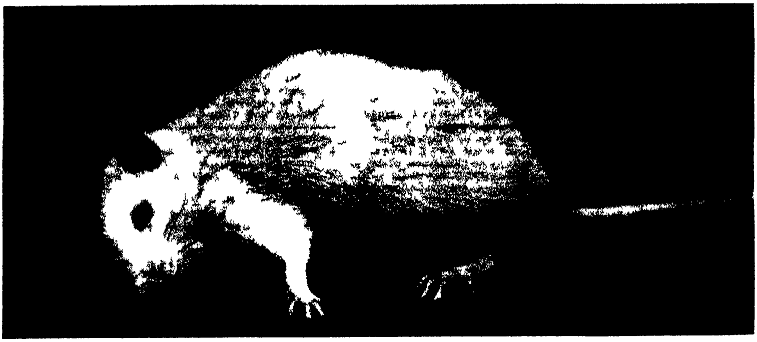

Fig III Rat 17 after 10 weeks on a basal diet supplemented with 20 mg of Adenine daily Weight, 77 g

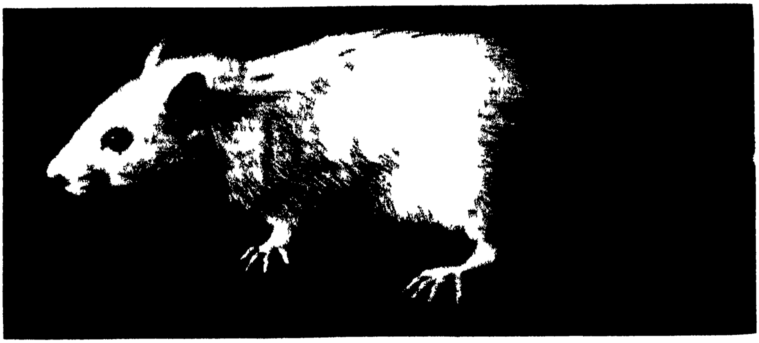

Fig IV The same Rat 10 days after receiving 0 4 g dry yeast daily Weight, 113 g

a daily dose of 20 mg, as the source of B_4. Some control animals fed on a basal diet supplemented with 5% dry yeast gave a satisfactory growth response within the test period, while both experimental animals died from B_4-deficiency after a few weeks feeding with adenine. In certain cases, when the animals began to decline in their body weights, the amount of B_1 and B_2 in the diet was doubled over a weekly period to make certain that the cessation of growth was not due to the insufficient supply of these Vitamins, but simply due to the ineffectiveness of adenine as the source of B_4.

The present author has also observed that the adenyl-thiomethyl-pentose, isolated by Prof. U. Suzuki, Ohdake and Mori(7) from brewers yeast has no B_4-activity.

Summary.

Pure Adenine, prepared from dry yeast does not serve as a source of Vitamin B_4 in the albino-rat. Since it has been shown repeatedly that the feeding of large amounts of adenine neither prevents nor cures the typical symptoms of Vitamin B_4 deficiency.

My thanks are due to Proffesor U. Suzuki for his advice and interest throughout the work to Dr. S. Ohdake for the supply of oryzanin, Vitamin B_1 concentrates and to Mr. H. Ariyama for his continued help in this experiment.

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On the Isolation of Apigenin from Manchurian Kaoliang (Andropogon sorghum, Brot).

Koji OKANO, Tadao ABE and Iwao OHARA.

(At the Central Laboratory of the South Manchuria Railway Co.)

(Received July 25, 1934)

The colouring matters of *Andropogon sorghum*, from which durasantalin ($Cl_6H_{12}O_5$) as a natural substantive dyestuff was obtained by A. G. perkin⁽¹⁾ (he employed var. vulgaris "red dura"), are now investigated and yellow needle crystals, melting at 347°C, are isolated. From the analytical numbers of the acetyl- and methyl-derivatives, the decomposition products and the absorption spectrum in ultra-violet rays of the crystal it is established that the substance is apigenin or 5, 7, 4′-trioxyflavone itself.

Experimental.

(1) Isolation of apigenin.

The material, the hulls of Manchurian Kaoliang [*Andropogon sorghum, Brot* (*Kaoliang*)] is digested with boiling methyl alcohol three times to extract the colouring matters yielding 7~8%. The deep purple-red concentrated extracts are poured into ether. After evaporation to dryness of ether apigenin is obtained from insoluble part in acetone as a fine purple-red mass. The yield is about 12% of all colouring matters, corresponding to about 1% of the raw materials. The acetone-soluble part or other colouring matters will be communicated in next paper.

For purification of apigenin, lead acetate is added in its alcohol solution to remove the impurities as precipitates. On evaporation of filtrates after treatment with SH_2, the minute straw-coloured crystals are separated. A small amount of calcium is so tightly adhesived to the above obtained crystals that is treated with concentrated HCl to remove and is sublimated in vacuum, then pure crystals are now collected.

(2) Analysis and derivatives of apigenin.

The apigenin thus obtained consists of pale yellow coloured needles, melting at 347°C, soluble in alcohol, methyl alcohol and pyridin. Its alcohol solution gives a purple-black colour with ferric chloride and dilute alkalis dissolve it with a yellow colouration.

Its derivatives, triacetyl, diacetyl, dimethyl and monomethyl, are now obtained by ordinary methods and those analytical numbers are shown as follows:

		apigenin	triacetyl 〃	diacetyl 〃	dimethyl 〃	monomethyl 〃
mel. pt. (°C)		347	185	198	172	168
C %	cal.	66.67	63.64	64.41	68.46	67.41
	obs.	66.65	62.59	64.30	68.32	67.42
H %	cal.	3.70	4 04	3.95	4.70	4 23
	obs.	3 94	4.04	3.85	4.71	4.32
methoxyl %	cal.				20.80	10.92
	obs.				20.36	10.28
mol. wt.	cal.		396	354	298	284
	obs.		364	341	313	262

The melting point of the triacetyl derivatives shows higher than that in the reports by Czajkowski⁽²⁾ or L. Schmidt⁽³⁾, which melts at 181~182°C, but others are identical.

(3) Decomposition of apigenin with alkalis.

(A) By digestion with the gentle action of alkalis (20% KOH for 2 hours), *p*-oxyacetophenone and phloroglucin are separated.

(B) Action of fused alkalis at 220~260°C results the formation of phloroglucin, *p*-oxybenzoic acid and acetic acid.

(4) The absorption spectrum of apigenin.

The absorption spectrum of apigenin in ultra-violet rays is now photographep and we observed its characteristic absorption, but in this paper it is spared.

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Investigation on Agar. (Continued). Physico-chemical Properties of Agar and their Influence on the Growth of Microorganisms.

By

Arao ITANO and Y. TUJI.

(Received July 10, 1934)

The previous reports(1, 2 & 3) dealt with the general review on agar as to the seaweeds from which the agar is manufactured and the iodine contents and also the history together with general chemical composition. In this investigation, the physico-chemical properties of agar of different grades I, II and III on the market, were determined and their subsequent influence on the growth of Azotobacter chroococcum, Bacillus subtilis and Saccharomyces cerevisiae was tried.

The following results were obtained: (1) Better the quality of agar is, contained less nitrogen and ash, and also faster the coagulation and solubility; (2) So far as the specific gravity, specific conductance, osmotic pressure and surface tension of the liquid agar and the filtered agar are concerned, better the quality of agar is, the value of these properties was smaller while the concentration of hydrogen ions of the first grade was greater than the others, and the unfiltered, liquefied agar was more acidic than the filtered agar.

The influence of agar of different grade, I and III was tried on Azotobacter chroococcum, B. subtilis and Saccharomyces cerevisiae, by cultivating them on the solid medium of known quality with an addition of I or III grade agar for each organism, namely Ashby's, Laurent's and the nutrient agar medium respectively. In all these cases, the organism grew better on the third grade agar and especially it was marked in case of Saccharomyces cerevisiae.

From the results thus obtained, it may be concluded that the agar of different grade differ markedly in regard to the physicochemical properties, and when they are used for cultivation of microorganisms as the solidifying agent, they influence the growth of microorganisms differently, and the agar of lower grade supports the better growth which may be due to the presence of a larger quantity of some stimulating substance such as iodine and others. Consequently it is very important to use the purified agar in order to obtain the uniform and comparative results in the microbiological investigation.

Reference.

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Untersuchungen über die Enzyme von Bombyx mori L. VII. Mitteilung.

Über die Eierkatalase. (1).

Von

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(Eingegangen am 31, Juli 1934)

Trotzdem zahlreiche Angaben über die in der Tierwelt vorhandene Katalase verliegen, haben wir dennoch kein eingehenden Arbeiten dieses Enzyms bei Insekten. Da ich einige Eigenschaften der Katalase aus den Eiern von Bombyx mori aufgeklart habe, schreibe ich hier einen deutschen Auszug aus dem japanischen Original, das im Journal of Agric. Chem. Soc. Jap. dargestellt wurde.

I. Gewinnung und Reinigung des Enzyms.

Ein Beispiel wird kurz angegeben: 850 g Eier wurden zerrieben, mit 500 ccm Wasser vermischt und 5 Stunden bei Zimmertemperatur belassen. Nach Absaugen wurden 750 ccm des Filtrates mit 370 ccm Alkohol versetzt und zentrifugiert. Die Restlosung wurde mit 270 ccm Chloroform geschüttelt und die Fällung durch Zentrifugieren abgetrennt. Zur wasserigen Losung wurden 300 ccm 1,5 proz. $Ca_3(PO_4)_2$-Suspension zugesetzt und nach kurzem Stehen wurde das Adsorbat gesammelt. Dann wurde die Katalase mit 100 ccm M/15-Na_2HPO_4-Lösung eluiert. Die von dem Adsorbens abzentrifugierte Katalaselosung wurde im Kollodiumsack 20 Stunden lang bei 0° dialysiert und unter Chloroformzusatz im Eisschrank aufbewahrt. Kat. f. der Eier wurde auf 7 bestimmt und die der gereinigten Enzymlösung auf 280.

II. Bestimmung der Enzymwirkung.

25 ccm 0,01 n-H_2O_2-Lösung, 1 ccm M/5-Phosphatpufferlösung von pH 6,8, Enzymlösung und Wasser wurden vermischt, und zwar betrug das Totalvolumen stets 30 ccm. Die Versuchstemperatur war 0°. Nach 0, 5, 10 und 15 Minuten wurden 5 ccm von der Reaktionsmischung abpipettiert und in 10 ccm 1 n-H_2SO_4 eingelassen. Dann wurde das nicht gespaltene H_2O_2 mit 0,005 n-$KMnO_4$-Lösung titriert. Die Aktivitat der Katalase wurde mit den nach der Formel für monomolekulare Reaktion berechneten Reaktionskonstanten verglichen.

III. Reaktionskonstanten und Enzymmengen.

Enzymmenge, E	1		4		8	
Zeit, Min.	$KMnO_4$, (ccm)	$k.10^4$	$KMnO_4$, (ccm)	$k.10^4$	$KMnO_4$, (ccm)	$k.10^4$
0	9,00	—	9,00	—	8,98	—
5	8,70	29	7,92	111	7,10	203
10	8,49	25	7,18	98	5,82	188
15	8,24	24	6,53	93	5,03	168
Mittel	—	26	—	101	—	186
$k.10^4/E$	—	26	—	25	—	23

IV. Einfluss der Wasserstoffionenkonzentration auf die Enzymwirkung.

pH	5,3	6,2	7,0	8,0
$k.10^4$	78	137	149	146
pH	6,6	6,8	7,2	7,7
$k.10^4$	149	167	157	151

V. Einfluss der Temperatur auf die Fnzymwirkung.

1) Optimale Temperatur.

Temp.	20	25	30	35
$k.10^4$	175	186	177	170

2) *Temperaturkoeffizient.*

Temp.	$k.10^4$	Temp.-Gebiet	$kt_{+10}:kt$	A
0	127	——	——	——
10	148	0~10	1,17	2426
20	166	10~20	1,12	1878
30	178	20~30	1,07	1202

Die Konstante A wurde nach der Arrheniusschen Formel $\ln(kt_1/kt_2)=A(T_1-T_2)/R\cdot T_1\cdot T_2$ berechtet.

VI. Thermostabilität des Enzyms.

1) *Tötungstemperatur.*

Erhitzungsdauer 60 Min., pH 6,8.

Erhitzungstemp.	Nicht erhitzt	50	55	60	70	80
$k.10^4$	145	94	75	39	15	7
Rel. k	100	65	52	27	10	5

2) *Abhängigkeit der Inaktivierung von der Enzymkonzentration.*

Erhitzungsdauer 60 Min., pH 6,8.

Verdünnnng	Nicht erhitzt	Erhitzte, Erhitzungstemp. 55°		
	1 1	1.1	1 5	1·9
$k.10^4$	136	78	75	54
Rel. k	100	57	55	40
$kc\ 10^4$	——	40	43	67

Die Inaktivierungskonstante kc wurde nach der Gleichung $kc=1/t\ \log(ka/kt)$ berechnet.

3) *Abhängigkeit der Inaktivierung von der Azidität.*

Erhitzungstemperatur 50°. Zeit 60 Min.

pH	$k.10^4$	pH	$k.10^4$
5,3	79	6,6	85
6,6	86	6,8	89
8,0	54	7,0	81

4) *Zeitlicher Verlauf der Inaktivierung.*

Erhitzungstemperatur 55°, pH 6,8.

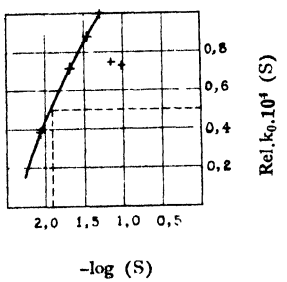
Erhits.-Dauer, Min	0	30	60	90
$k.10^4$	113	75	59	49
Rel.k	100	66	52	43
$kc.10^4$	——	59	47	40

VII. Affinitätskonstante der Verbindung Enzym-substrat.

(S), m	-log (S)	$k_0.10^4$	$k_0.10^4$. (S)	Rel. $k_0.10^4$. (S)
0,0086	2,066	198	1,70	0,38
0,0206	1,686	152	3,13	0,71
0,0344	1,463	111	3,82	0,87
0,0495	1,305	89	4,41	1,00
0,0698	1,156	47	3,28	0,74
0,0942	1,026	33	3,21	0,73

(S) bedeutet die Konzentration des Substrats und k_0 die Reaktionskonstante in der Zeit 0.

Aktivitäts-pS-Kurve



log Km = −1,925

Km = 0 0119

1/Km ≑ 84

Zusammenfassung.

(1) Unter Anwendung der Adsorptionsmethode mit Hilfe des Calciumphosphats wurde aus den Eiern von Bombyx mori ein Katalasepraparat hergestellt.

(2) Die monomolekularen Reaktionskonstanten der Katalasewirkung in 0,01 n-H_2O_2-Lösung bei 0° fallen allmahlich. Aber zwischen den Enzymmengen und Reaktionskonstanten besteht eine gute Proportionalität.

(3) Die Eierkatalase wirkt am besten bei pH 6,8; ihre Optimaltemperatur liegt bei 25°.

(4) Der Temperaturkoeffizient $kt_{+10}:kt$ und die Konstante A der Arrheniusschen Temperaturformel zwischen 0 bis 10° sind 1,17 und 2400. Bei höherer Temperatur nehmen diese Werte ab.

(5) Die Tötungstemperatur der Katalase liegt bei etwa 55°. Nach 60 Minuten Erhitzung bei 80° ist die Aktivität nahezu auf Null gesunken.

(6) Die Katalase zeigt die maximale Thermostabilität bei pH 6,8 und diese Stabilität nimmt bei steigender Enzymkonzentration zu.

(7) Die Inaktivierung der Eierkatalase bei Erhitzung verläuft nicht monomolekular, sondern die Konstante kc der monomolekularen Reaktion nimmt ab.

(8) Als Affinitatskonstante für die Verbindung Enzym-Substrat wurde 84 bestimmt.

Untersuchungen uber die Enzyme von Bombyx mori L. VIII. Mitteilung.

Über die Eierkatalase (2).

Von

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(Eingegangen am 31, Juli 1934)

Bezüglich der Einflüsse der ausseren Faktoren aus die Katalasewirkung ist bereits von zahlreichen Forschern mitgeteilt worden. Um eingehendere Kenntnisse über die Hemmungsrescheinungen der Katalase durch chemische Stoffe zu gewinnen, habe ich nun die Salzwirkung auf das Enzym studiert.

I. Gewinnung und Reinigung des Enzyms.

800 g der fein gemahlenen Eier wurden mit 400 ccm Wasser 3 Stunden lang bei Zimmertemperatur extrahiert. Der Extrakt wurde uber Nacht unter Chloroformzusatz im Eisschrank aufbewahrt und dann mit 250 ccm Alkohol und 200 ccm Chloroform unter Kuhlung kraftig geschüttelt. Nach Abzentrifugieren der Fällung wurde die wasserige Losung mit 300 ccm 3 proz. $Al(OH)_3$-Suspension, die nach der Vorschrift von Willstatter und Racke (Liebigs Ann. 425, 1.) bereitet wurde, versetzt. Die 150 ccm betragende Elution, welche mit 1 proz. sekundarem Natriumphosphat erhalten worden war, wurde wahrend 1 Tages im Eisscharnk dialysiert. Die Eier zeigten Kat. f. etwa 7 und die gereinigte Katalaselosung 270.

II. Einrichtung der Hemmungsversuche.

Die Versuche wurden mit Reaktionsmischungen von 30 ccm Totalvolumen ausgeführt. 2,5 ccm 0,1 n-H_2O_2, 1 ccm M/5-Phosphatpuffer von pH 6,8, 1 ccm Enzymlösung und Salzlosung wurden vermischt und mit Wasser auf 30 ccm aufgefüllt. Die Versuchstemperatur wurde stets auf 0° gehalten. Zur Bestimmung der Katalaseaktivitat wurde wie früher die Permanganatmethode angewandt, und auch als Mass der Hemmung die Reaktionskonstante erster Ordnung gewahlt.

III. Hemmung durch Natriumsalze.

	Salzkonz. n	0	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}
NaCl	k.10^4	160	—	164	156	152	119	112
	Rel.k	100	—	102	97	95	74	70
NaF	k 10^4	158	—	—	—	135	70	15
	Rel.k	100	—	—	—	85	44	9
$NaNO_3$	k 10^4	158	142	—	111	—	94	48
	Rel k	100	90	—	71	—	60	31
Na_2SO_4	k.10^4	155	155	—	154	—	150	—
	Rel k	100	100	—	100	—	95	—
Na_3PO_4	k 10^4	151	—	153	—	148	—	146
	Rel k	100	—	101	—	98	—	96
Na_2S	k.10^4	167	130	76	10	—	—	—
	Rel.k	100	77	45	6	—	—	—
NaSH	k 10^4	173	—	90	19	14	—	—
	Rel k	100	—	52	11	8	—	—
NaCN	k 10^4	145	132	19	3	—	—	—
	Rel.k	100	84	13	2	—	—	—
Na Azetat	k.10^4	162	130	—	127	—	121	62
	Rel.k	100	80	—	78	—	75	44
Na-Butyrat	k 10^4	140	—	—	145	146	134	—
	Rel k	100	—	—	104	104	96	—
Na-Lactat	k 10^4	154	141	—	133	—	124	80
	Rel.k	100	92	—	86	—	80	52
Na-Oleat	k.10^4	151	131	96	42	18	—	—
	Rel.k	100	87	64	28	12	—	—

Aus den oben erwähnten und anderen Data wurden durch graphische Intrapolation diejenigen Konzentrationen der Salze ermittelt, die die Katalasewirkung auf die Halfte herabsetzen ($H_{50\%}$).

Salz	$H_{50\%}$, n	Salz	$H_{50\%}$, n	Salz	$H_{50\%}$, n
Butyrat	$> 10^{-2}$	Lactat	10^{-1}	Oleat	5.10^{-5}
Sulfat	$>8.10^{-1}$	Azetat	8.10^{-2}	Hydrosulfid	10^{-5}
Phosphat	7.10 ^{1}	Nitrat	2.10^{-2}	Sulfid	9.10^{-6}
Chlorid	6.10^{-1}	Fluorid	8.10^{-3}	Cyanid	3.10^{-6}

IV. Hemmung durch Kaliumsalze.

	Salzkonz. n	0	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}
KCl	$k.10^4$	149	—	—	133	—	105	100
	Rel.k	100	—	—	89	—	70	67
KNO_3	$k.10^4$	149	149	—	122	—	84	39
	Rel.k	100	100	—	82	—	56	27
K_2SO_4	$k.10^4$	135	136	—	136	—	—	125
	Rel.k	100	100	—	100	—	—	93
K_3PO_4	$k.10^4$	152	—	153	—	148	—	136
	Rel.k	100	—	100	—	97	—	89
K_2S	$k.10^4$	136	123	85	25	—	—	—
	Rel.k	100	90	63	19	—	—	—
KCN	$k.10^4$	130	118	12	3	—	—	—
	Rel.k	100	91	9	2	—	—	—
K-Azetat	$k.10^4$	147	—	148	—	148	112	49
	Rel.k	100	—	100	—	100	76	33
K-Butyrat	$k.10^4$	145	—	—	139	136	134	—
	Rel.k	100	—	—	96	94	92	—

Salz	$H_{50\%}$, n	Salz	$H_{50\%}$, n
Butyrat	>3 10 ^{2}	Azetat	6.10^{-2}
Sulfat	$>6.10^{-1}$	Nitrat	3.10^{-2}
Phosphat	7.10 ^{1}	Sulfid	4 10^{-5}
Chlorid	4 10^{-1}	Cyanid	5 10^{-6}

Zusammenfassung.

Es wurden die hemmende Wirkungen den verschiedenen Natrium- oder Kaliumsalze auf die durch Tonerdeadsorption gereinigte Eierkatalase geprüft. Dabei ergab sich, dass der Effekt der Salze hauptsachlich auf ihre Anionen zurückzuführen ist und zwar die folgende Reihenfolge von schwacher zu starker Hemmung besteht: Butyrat$<SO_4<PO_4<Cl<$Lactat$<$Azetat$<NO_3<F<$Oleat$<SH<S<CN$.

Untersuchungen über die Enzyme von Bombyx mori L. IX. Mitteilung.

Über die Proteasen und Amylase des Blutes (2).

Von

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(Eingegangen am 31, Juli 1934.)

Obwohl die eiweissspaltende Reaktion des Blutes der Seidenraupe zwei ausgeprägte pH-Optima zeigt, so ist noch nicht einwandfrei festgestellt worden, dass im Blute derselben zwei Sorten Proteasen, namlich Pepsinase und Tryptase vorkommen (Vgl. dieses Bull. 9, 19.). Deshalb wurden in der vorliegenden Arbeit vorlaufig die Namen Protease-a und Protease-b gebraucht, wobei das Wirkungsoptimum der ersteren bei pH 2,3 liegt und das der letzteren bei pH 8,8.

I. Proteasen.

A. Protease–a.

Die Versuchsanordnung wurde diesmal gegenüber der früher von mir beschriebenen nur unwesentlich verandert. Die Substratlösung wurde dadurch bereitet, dass 5 g Kasein nach Hammersten mit 100 ccm 0,1 n-NaOH gelöst und mit Wasser auf 1000 ccm gefüllt wurden. Die Reaktion der Proben wurde mit 0,1 m-Sörensenschem Zitrat-HCl-Puffer wahrend der Versuchszeit konstant gehalten. Als Enymlosung benutzte ich das mit 0,85 proz. NaCl-Lösung fünffach verdünnte Blut. 4 ccm 0,5 proz. Kaseinlösung, 5 ccm Pufferlösung von pH 2,3 und 1 ccm Enzymlösung wurden gemischt und während 24 Stunden bei 30° stehen gelassen. Die Ermittlung des Grades der Kaseinspaltung durch das Blut führte ich nach der einfachen nephelometrischen Methode durch. Zu diesem Zweck wurde in den meisten Fällen 0,2 ccm von der Reaktionsmischung entnommen und in 5 ccm 5 proz. Sulfosalicylsäure eingelassen. Die entstandene Trübung wurde mit der Standardtrübung, die durch Hinzusetzen der nicht gespaltenen Kaseinlösung zur 5 proz. Sulfosalicylsäure hergestellt wurde, mittels des Nephelometers verglichen. Zugleich wurden Blindproben ausgeführt.

1) *Änderungen durch Hungerzustand bei den Larven.*

Hungerstunden	♀		♂	
	Gespaltenes Kasein mg	Spaltung %	Gespaltenes Kasein mg	Spaltung %
0	8,98	44,9	10,00	50,0
24	7,72	38,6	8,04	40,2
48	6,28	31,4	6,00	30,0

2) *Unterschied zwischen gut gewachsenen und schlecht gewachsenen Larven.*

	Gut gewachs. Larven		Schlecht gewachs. Larven	
	♀	♂	♀	♂
Gespaltenes Kasein, mg	8,89	9,55	9,23	9,63
Spaltung, %	44,5	47,8	46,2	48,2

3) *Veränderungen im Laufe der drei Entwicklungsperioden von Bombyx mori.*

	Tage		♀		♂	
			Gespalt. Kasein mg	Spaltung %	Gespalt. Kasein mg	Spaltung %
Larve	V. Lebensalter	3	5,71	28,6	5,57	27,9
		6	7,61	38,1	8,62	43,1
		8	9,63	48,2	10,34	51,7
Einspinnen des Kokons		2	10,34	51,7	10,48	52,4
		4	8,24	41,2	8,43	42,4
Puppe		2	6,92	34,6	8,89	44,5
		6	8,71	43,6	9,71	48,6
		9	12,51	62,6	13,68	68,4
		11	11,36	56,8	13,33	66,7
Schmetterling		2	8,43	42,2	——	——

4) *Unterschied zwischen den verschiedenen Rasen der Seidenraupen.*

V. Lebensalter, Tage	3		5		8	
Rasse	Gespalt. Kasein mg	Spalt. %	Gespalt. Kasein mg	Spalt. %	Gespalt. Kasein mg	Spalt. %
Japan-111-E	6,48	32,4	10,24	51,6	10,28	51,4
Japan-110-G	5,64	28,3	8,12	40,6	9,99	50,0

China-7-B	9,01	45,1	10,87	54,4	11,83	59,2
China-7-D	9,13	45,7	9,19	46,0	11,23	56,2
Europa-7-A	7,04	35,2	9,79	49,0	9,35	46,8
Europa-7-C	9,24	46,2	10,76	53,8	11,10	55,6

B. Protease-b.

Die Versuche erfolgten mittels folgenden Verfahrens. Zur Mischung der 4 ccm 0,5 proz. Kaseinlösung und der 5 ccm M/15-Glykokoll-NaCl-NaOH-Pufferlösung von pH 8,9 wurde 1 ccm mit 0,85 proz. NaCl-Lösung fünffach verdünntes Blut hinzugefügt. Nach 24 Stunden Erwärmung bei 30° wurde wie früher die Abspaltung des Kaseins nephelometrisch ermittelt.

1) *Änderungen durch Hungerzustand bei den Larven.*

Hungerstunden	♀		♂	
	Gespaltenes Kasein mg	Spaltung %	Gespaltenes Kasein mg	Spaltung %
0	1,82	9,1	2,72	13,6
24	0,82	4,1	1,58	7,9
48	1,58	7,9	2,50	12,5

2) *Unterschied zwischen gut gewachsenen und schlecht gewachsenen Larven.*

	Gut gewachs Larve		Schlecht gewachs Larve	
	♀	♂	♀	♂
Gespaltenes Kasein, mg	2,28	1,58	2,72	2,93
Spaltung, %	11,4	7,9	13,6	14,7

3) *Veränderungen im Laufe der drei Entwicklungsperioden von Bombyx mori.*

	Tage		♀		♂	
			Gespalt. Kasein mg	Spalt %	Gespalt Kasein mg	Spalt %
Larve	V. Lebensalter	3	8,33	41,7	8,43	42,2
		6	5,57	27,9	6,00	30,0
		8	3,72	18,6	5,71	28,6
Einspinnen des Kokons	2		2,28	11,4	3,53	17,7
	4		4,95	24,8	4,44	22,2

Puppe	2	4,95	24,8	4,95	24,8
	6	5,42	27,1	5,57	27,9
	9	7,61	38,1	7,61	38,1
	11	8,71	43,6	8,89	44,5
Schmetterling	2	2,50	12,5	——	——

4) *Unterschied zwichen den verschiedenen Rassen der Seidenraupens.*

V Lebensalter, Tage	3		5		8	
Rasse	Gespalt. Kasein mg	Spalt. %	Gespalt. Kasein mg	Spalt. %	Gespalt. Kasein mg	Spalt. %
Japan-110-E	5,55	27,8	5,72	28,6	5,26	26,3
Japan-110-G	4,18	21,0	4,08	20,5	5,71	28 6
China-7-B	8,38	42,0	5,79	29,0	4,72	23,6
China-7-D	7,16	35,8	5,69	28,5	4,98	24 9
Europa-7-A	5,71	28,6	4,36	21,8	3,82	19,6
Europa-7-C	7,04	35,2	4,44	22,2	4,44	22,2

II. Amylase.

Zur Bestimmung der saccharifizierenden Wirkung des Blutes wurde immer 1 ccm mit 0,85 proz. NaCl-Lösung fünffach verdünntes Blut mit 5 ccm 5 proz. frisch hergestellter Stärkelösung und mit 1 ccm 0,2 m-Phosphatpuffer von 6,8 versetzt, mit Toluol überschichtet und für 24 Stunden in einen Thermostaten bei 30° gestellt. Nach Verlauf dieser Zeit wurde die Zuckerbestimmung nach Bertrand ausgeführt. Die Zahlen, durch die wie das Verzuckerungsvermögen des Blutes ausdrücken, bedeuten die Milligramme Zucker, die als Maltose berechnet werden. Der Versuch wurde auf jeden Fall am Tage der Blutentnahme angestellt.

1) *Änderungen durch Hungerzustand bei den Larven.*

Hungerstunden	♀		♂	
	Zucker, mg	Spaltung, %	Zucker, mg	Spaltung, %
0	32,2	12,2	31,5	12,0
24	46,5	17,7	36,8	14,0
48	45,0	17,1	44,1	16,8

2) *Unterschied zwischen gut gewachsenen und schlecht gewachsenen Larven.*

	Gut gewachs. Larve		Schlecht gewachs. Larve	
	♀	♂	♀	♂
Zucker mg	37,3	31,9	36,8	31,5
Spaltung, %	14,2	12,1	14,0	12,0

3) *Veränderungen im Laufe der drei Entwicklungsperioden von Bombyx mori.*

	Tage		♀		♂	
			Zucker, mg	Spalt %	Zucker, mg	Spalt %
Larve	V. Lebensalter	3	25,3	9,6	25,3	9,6
		5	33,9	12,9	31,5	12,0
		8	49,5	18,8	37,0	14,1
Einspinnen des Kokons	3		57,3	21,8	50,8	19,3
	5		54,7	20,8	53,9	20,5
Puppe	4		44,3	16,8	42,6	16,2
	8		41,3	15,7	35,0	13,3
	11		39,9	15,2	31,1	11,8
	13		30,8	11,7	25,6	9,3
Schmetterling	2		42,3	16,1	—	—

4) *Unterschied zwischen den verschiedenen Rassen der Seidenraupen.*

V. Lebensalter, Tage	3		5		8	
Rasse	Zucker mg	Spalt %	Zucker mg	Spalt %	Zucker mg	Spalt %
Japan 110 I	13,5	5,1	17,5	6,6	20,8	8,0
Japan-110 G	20,3	7,6	25,5	9,7	27,3	10,4
China-7-B	25,3	9,6	32,7	12,5	43,3	16,5
China-7-D	17,6	6,2	24,9	9,5	28,2	10,7
Europa-7-A	25,5	9,7	32,3	12,3	37,4	14,2
Europa-7-C	27,0	10,3	33,1	12,6	47,3	18,0

III. Diskussion.

Durch eine Reihe früher mitgeteilter Versuche, die Änderungen des Fermentgehaltes des Blutes bei den verschiedenen physiologischen sowie pathologischen Bedingungen von Bombyx mori zum Gegenstand haben, dürfte die Abhangigkeit einiger Blutfermente vom Stoffwechsel in eindeutiger Weise festgestellt sein. Es konnte namlich vor allem in den fünften und sechsten Mitteilungen gezeigt werden, dass die Saccharase, Maltase und Lipase im Blute dieses Insekts bei den Umsetzungen der Disaccharosen und des Fettes in seinem Körper eine wichtige Rolle spielen.

Diese Kenntnis enger Beziehungen dieser drei Bluthydrolasen zu den Umwandlungsvorgangen der Stoffe im Körper hat mich weiterhin zu der Vermutung geführt, dass auch die Amylase und Proteasen des Blutes bei Bombyx mori am Kohlenhydrat- bzw. Eiweissstoffwechsel Anteil nehmen müssen. Zu Gunsten dieser Vorstellung scheinen mir die in der vorliegenden Arbeit angestellten Experimente zu sprechen.

Ich beobachtete, dass die Rassen, in deren Digestionssaft keinen oder nur wenige Amylase vorkommt, recht viel starkeres amylolytisches Ferment im Blute enthalten, als diejenigen Rassen, die eine viel grossere starkespaltend Kraft der Verdaungsflüssigkeit besitzen. Es erscheint biologisch ausserst unwahrscheinlich, dass selbst bei der Seidenraupe ihre Magenwand hochmolekulare Nahrstoffe, wie Starke oder Eiweiss, durchlassen sollte. Aber auf Grund meiner zahlreichen Untersuchungen über dieses Insekt durfte die Moglichkeit gegeben sein, dass wenigstens die Abbauprodukte, die aus solchen Nahrungsstoffen im ersten Stadium der Verdauung entstehen, durch die Wand des Digestionskanals der Seidenraupe in das Blut derselben kommen und dort durch die Blutfermente weiter gespalten werden.

Von grossem Interesse ist die Veranderung der Blutamylase im Laufe der drei Entwicklungsperioden von Bombyx mori. Eine tagliche, verhaltnismassig erhebliche Zunahme an Amylaseaktivitat erfolgt wahrend des funften Lebensalters der Larve, und diese Zunahme erreicht in der Zeit des Spinnens des Kokons und der Umwandlung der Larve zur Puppe ein Maximum. Dagegen beobachtet man eine ausgesprochene, allmahliche Abnahme an Tatigkeit der Blutamylase wahrend der Puppenperiode Was die bei den Schmetterlingen bestimmten amylolytischen Wirkungen des Blutes betrifft, so ist diese viel grosser als die am Ende des Puppenstadiums bestimmten.

Ferner fand ich bestatigt, dass der Hungerzustand bei der Larve eine bedeutende Vermehrung der Amylase hervorbringt.

Es scheint also sehr wahrscheinlich, dass mit den Intensitatsschwankungen des Energiewechsels, der von der Umsetzung des Korperglykogens seinen Ursprung nimmt, auch diese gesetzmassigen Schwankungen des Amylasegehaltes bei Metamorphose bzw. bei Hunger parallel gehen.

Aus den oben erwahnten Data gelange ich zu der Schlussfolgerung, dass die Blutamylase bei Bombyx mori an der Zersetzung des Glykogens und auch zum Teil an der Verdauung der in den Maulbeerblattern enthaltenen Stärke beteiligt ist.

Sehr kompliziert ist das Verhalten der Proteasen. Trotzdem die Wasserstoffionenkonzentration des Blutes von Bombyx mori nahe bei pH 6,8 liegt, zeigt die darin vorkommende Protease zwei pH-Optima, nämlich pH 2,3 und 8,8. Dies ist eine merkwürdige Tatsache. Vielleicht eine der physiologischen Funktionen der Protease-a liegt, wie mir vorkommt, darin, dass dieses Ferment eine Unvollkommenheit der Eiweissverdauung durch den Digestionssaft ergänzt; denn die Protease dieses Saftes wirkt nur bei starker alkalischer Reaktion.

Da sich die Wirkungen der Blutproteasen mit der Metamorphose von Bombyx mori und beim Hunger der Larve regelmässig verändern, scheinen diese Fermente am Eiweissstoffwechsel in ihrem Körper Anteil zu nehmen.

Wenn wir die erhaltenen zahlreichen Daten in Beziehung bringen zu den morphologischen Verwandlungen, die in den verschiedenen Entwicklungsperioden eintreten, dann können wir über das, was wir beobachtet haben, eine interessante Diskussion eröffnen.

Bei Bombyx mori besteht ein grosser Unterschied zwischen der Körperkonstruktion der Larve und der des Schmetterlings. Heftige Umformungen der verschiedenen Gewebe oder Organe müssen infolgedessen während der Zeit der Puppe stattfinden. Nach meinen Erfahrungen scheinen Fermente im Blute mit diesen Umformungen zu tun zu haben. Vor allen Dingen kommen hierbei die Änderungen der Proteasen im Laufe der Entwicklung in Betracht. Denn die Zersetzung bzw. Synthese des Eiweiss ist mit der Auflösung bzw. Neubildung der Organzellen verknüpft. Meine Versuchsresultate weisen darauf hin, dass die Wirkung der Protease-a, deren optimale Wasserstoffionenkonzentration bei pH 2,3 liegt, vom ersten Stadium des fünften Lebensalters bis zur Zeit des Kokonspinnens nach und nach zunimmt und dann sinkt, und ferner, dass im ganzen Zeitraum der Entwicklung diese Fermentwirkung einige Tage vor dem Ausschlüpfen der Imagines am aktivesten ist und dann wieder allmählich sich verringert. Etwas anders verhält sich die Protease-b. Dieses Ferment, welches bei pH 8,8 am besten wirkt, wird während des fünften Lebensalters der Larve von Tag zu Tag schwächer, erreicht sein Minimum in den Tagen des Kokonspinnens, und verstärkt sich dann kurz vor dem Schlüpfen bis zu einem Maximum.

Charakteristisch ist die Erscheinung, dass eine kurz vor dem Schlüpfen stehende Puppe solche maximale Wirkungen beider Proteasen aufweist. Diese Erscheinung, welche wir niemals mit anderen Blutfermenten von Bombyx mori erfahren können, gibt einen wesentlichen Stützpunkt für die Annahme einer Abhängigkeit der Blutproteasen von der Ausbildung der Geschlechtsprodukte bei den Insekten ab.

Es ist aber schwer, den Zusammenhang zwischen den Blutfermenten und den Organumformungen bei Bombyx mori genau zu bestimmen, weil keine eingehenden Arbeiten über den Verlauf der histologischen Veranderungen wahrend der Entwicklung dieses Insekts vorhanden sind.

Die in dieser Untersuchungsreihe über die Enzyme von Bombyx mori mir oft begegneten Befunde, dass einige Fermente des Blutes, wie zum Beispiel Saccharase, Maltase, Lipase und Protease, bei schlecht gewachsenen Larven etwas starker sind als bei gut gewachsenen, sind meiner Ansicht nach durch zwei Arten von Tatsachen zu erklaren, namlich erstens durch die Ergebnisse meiner zahlreichen Versuche, dass die Blutfermente bei diesem Insekte an den Stoffwechselprozessen in seinem Korper beteiligt sind, und zweitens durch das im allgemeinen bekannte Oberflachengesetz, nach welchem der Energiebedarf bei Tieren sich nach ihren Oberflachenentwicklung richtet.

Immerhin zeigen alle diese Untersuchungen, insbesondere die Versuche, die die Wirkungen der Blutfermente bei Bombyx mori in den verschiedenen Phasen ihrer Entwicklung betreffen, eine Mengen Anhaltspunkte, die für wichtige Funktionen der Oxydationsfermente bei den Stoffwechselvorgangen im Organismus und auch für gesetzmassige Beziehungen dieser Oxydationsfermente und der hydrolytischen Fermente im Korper sprechen. Die Versuche sollen in dieser Richtung sowie auch noch in anderer Beziehung fortgesetzt werden.

Zusammenfassung.

(1) Die Wirkungen der Proteasen im Blute sind bei den Mannchen etwas starker als bei den Weibchen und auch bei schlecht gewachsenen Larven ziemlich hoher als bei gut gewachsenen. Hungerzustand bei Larven ruft Abnahme der Proteasenaktivitat hervor. Im allgemeinen ist der Proteasengehalt des Blutes der chinesischen und der europaischen Rassen etwas hoher als derjenige der japanischen.

(2) Es wurden die Veranderungen der Blutfermente im Laufe der drei Entwicklungsperioden verfolgt. Die Protease-a, die bei pH 2,3 die optimale Aktivitat zeigt, wird wahrend des fünften Lebensalters der Larve mit dem Wachstum vermehrt und in den Tagen des Kokonspinnens sehr aktive. Dann sinkt sie und nimmt wieder zu, um ihr Maximum kurz vor dem Ausschlüpfen der Imagines zu erreichen. Hierauf verringert sie sich am Ende der Puppenperiode und im Schmetterlingsstadium allmahlich.

Aber die Protease-b, deren optimale Wirkung bei pH 8,8 liegt, wird im fünften Lebensalter nach und nach schwacher und erreicht ihr Minimum etwa in der Zeit des Spinnens des Kokons. Dann vermehrt sie sich gegen Ende der Puppenzeit bis zu einem Maximum und fallt in der Imago wieder ab.

Die eiweissspaltende Kraft der Protease-a ist stets recht viel grösser als diejenige der Protease-b.

(3) Die Wirkung der Blutamylase ist bei den Weibchen immer starker als bei den Mannchen, aber es besteht kein wesentlicher Unterschied zwischen gut gewachsenen und schlecht gewachsenen Larven. Durch Hunger wird die Amylase gesteigert. Die Amylaseaktivitat der chinesischen und der europäischen Rassen ist höher als diejenige der japanischen.

Die Amylasewirkung nimmt im fünften Lebensalter der Larve von Tag zu Tag zu und erreicht in der Zeit des Kokonspinnens und des Verpuppens ein Maximum. Wahrend der Periode der Puppenruhe vermindert sich die Schmetterlingsperiode wird sie wieder bedeutend starker.

(4) Es wurde über die physiologische Bedeutung der Protease und Amylase des Blutes bei Bombyx mori diskutiert und dabei zu der Schlussfolgerung geführt, dass diese Blutfermente in den Stoffwechselvorgangen und auch zum Teil in den Verdauungsprozessen bei diesem Insekte eine wichtige Rolle spielen.

Grossen Dank schulde ich Herrn Prof. Y. Okuda, der mich immer in liebenswürdiger Weise mit Rat und Tat unterstützte.

Über die physiko-chemiche Eigenschaft des Harnstoffnitrats.

Von

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(Eingegangen am 2, August 1934)

Harnstoffnitrat, das aus je ein Molekül von Harnstoff und Salpetersäure besteht, hat nach mehrere Versuche sich als eine geeignete Düngemittel erwiesen, enthaltend das Stickstoff von Harnstoff- bzw. Salpeterformen. Seine augenscheinliche Überlegenheit dem Harnstoff ist seine kleinere Hygroskopicität. Wegen des leichten Darstellung und Behandlung gibt es solche Hoffnung für diese Verbindung in der Zukunft, dass sie auch als Düngemittel Verwendung finden mag.

Es wurde in vorliegender Arbeit über die verschiedene wichtige Eigenschaften des Harnstoffnitrats studiert.

(1) Wegen kleiner Löslichkeit des Harnstoffnitrats in Salpetersäure ist Harnstoffnitrat aus der gesättigten Wasserlösung Harnstoffes und entsprechenden Menge von konz. Salpetersaure ganz einfach als die Niederschlage zu erhalten.

(2) Der Schmelzpunkt ist 157°C, und das spezifische Gewicht betragt 1,63.

(3) Die Löslichkeit des Harnstoffnitrats in verschiedenen Losungsmitteln ist unten angegeben:

a) Loslichkeit des Harnstoffnitrats in Wasser, Ethanol und Methanol.

(Harnsffnitrat Gr. in 100 Gr. Losung)

	Wasser	Methanol	Ethanol
30°C	26,28	11,65	5,05
35°C	30,01	13,58	6,18
40°C	34,94	15,78	7,46

b) Loslichkeit des Harnstoffnitrats in Salpetersaure.

Konz. von HNO_3 in *N*.	0,1	0,2	0,5	1,0
Löslichkeit	24,7	22,0	18,5	15,6

c) Loslichkeit des Harnstoffnitrats in anderen Losungsmitteln.

Diese Verbindung ist gar nicht loslich in Äther, Benzol und Chloroform bei gewohnlicher Temperatur wie bei dem Harnstoff. Dagegen in Azeton bei 30°C ist sie 3,7% löslich.

(4) Die Zahigkeit der Harnstoffnitratlosung im Wasser.

Die Zahigkeit wurde mit Ostwaldviskosimeter gemessen, und als die spezifische Zahigkeit gegen Wasser (=1) angegeben.

a) Zahigkeit der Harnstoffnitratlosung bei verschiedenen Konzentrationen (30°C).

Konz. d. Lösung in Mol.	1,827	1,096	0,914	0,609
Zähigkeit	1,192	1,102	1,065	1,039

b) Zahigkeit der gesattigten Harnstoffnitratlosung bei verschiedenen Temperaturen.

Temp. °C	25	30	35	40	45
Zähigkeit	1,157	1,192	1,236	1,286	1,338

(5) Der Wasserdampfdruck der Harnstoffnitratlösung im Wasser.

Der Wasserdampfdruck der gesattigten Harnstoffnitratlösung im Wasser wurde mittels Isoteniskops ermittelt, und mit dem der Harnstofflosung verglichen. Dadurch hat man die kleinere Hygroskopicitat Harnstoffnitrats gegenüber Harnstoff bestatigt.

Temp. °C	Wasserdampfdruck in mm Hg.	
	Harnstoffnitrat	Harnstoff
30	29,1	23,1
35	38,2	29,0
40	49,8	37.7

Studies on Dietary Requirements for Lactation.

II.—Presence of Lactation Promoting Substance in Liver Extract.

By

Waro NAKAHARA and Fumito INUKAI.

[Reprinted from Sci. Pap. I. P. C. R., Vol. 22, pp. 33—38, 1934]

Introduction.

We previously described a synthetic diet which is adequate for excellent growth of young rats, maintenance of good general health, pregnancy and parturition, but is inadequate for successful lactation.(1) This diet has been worked out in order to provide a basal diet for animal experiments aiming at the elucidation of the dietary requirement for lactation, for it is obvious that such a diet as this is prerequisite for a proper approach to the subject. The lactation promoting action of any substance may now be tested by simply adding it to this basal diet.

In the present paper we wish to report our subsequent experiment, which, in our opinion, demonstrates the presence of a lactation promoting substance in liver extract.

Basal Diet and Liver Extract.

The composition of the basal diet in question is as follows:—

Polished rice powder	75 g
Fish protein	10 g
Batter	10 g
McCollum's salt mixture	5 g
Dried yeast	5 g

Liver extract was prepared as follows: 1 kg of mashed fresh beef liver was throughly mixed with water, to which 50 c.c. of 25% sulphuric acid and sufficient amount of 90% ethyl alcohol were added to make the total volume of this mixture 5 litres and the final concentration of alcohol 60%. The extract was pressed out of the mixture, decolorized by means of a small amount of animal charcoal, and neutralized with calcium oxide. It was then concentrated into a suitable volume in the atmosphere of CO_2 gas under reduced pressure and at a temperature below 60°C.

The concentrated extract thus prepared was added to the basal diet at the rate of 3 g per 100 g of basal diet. This amount of extract corresponds to the yield from 100 g of fresh liver.

Breeding Experiment.

The general procedure of the present experiment was the same as that adopted in our previous work. A series of pairs (males and females) of young albino rats, each pair in a separate cage, were maintained on the basal diet, keeping careful observations on the body weight changes, especially of the females. Fifty-eight days after the beginning of the feeding, liver extract was added to the diet for the test group while the control group was continued on the basal diet alone.

The parturition was anticipated by means of observations on growth curves and the general appearance of the females, without having recource to the examination of vaginal smears. As soon as the approaching parturition was detected, the cohabitant male was separated and appropriate quantities of straw and cotton were put in the cage. Feeding and observation were continued with minimum disturbance to the gravid female. The same care was exercised after parturition, and all unnecessary disturbance to the mother rat and her young was avoided.

The success or failure of lactation was determinable within two or three days after parturition. The females suckling the young were continued under observation for three weeks more, i. e., up to the time of weaning the young, and at that time the body weight of the young was noted.

In the case of the failure to suckle the young the mother rat was killed as soon after the death of the young as possible (always within 3 days after parturition), and autopsy was performed in order to ascertain the functional state of the mammary grands.

The results of the experiment are shown in Charts 1 and 2.

Chart 1 represents the growth curves of 6 females composing the test group, which was given supplementary feeding of liver extract. The beginning of this supplementary feeding is marked by the sign +.

P indicates parturition, and the number of black dots following it represent the number of young born. The young which were successfully suckled are indicated by short growth curves starting from the black dots, the terminal point of the growth curve being the body weight at the age of 21 days.

As is evident from the chart, 3 out of 6 females suckled their young and were able to raise them.

The remaining 3, which failed to suckle their young, were killed and their mammary glands examined. It is of importance to note that all these rats showed evidence of active milk secretion, as we shall describe later in this paper.

Chart 2 shows growth curves of 4 control females which were maintained on the basal diet alone throughout. None of these females suckled their young, as may be expected from the result of our previous experiments. Moreover, anatomical examination established that the failure of these control females to suckle their young was directly ascribable to the lack of milk secretion (See a subsequent section of this paper).

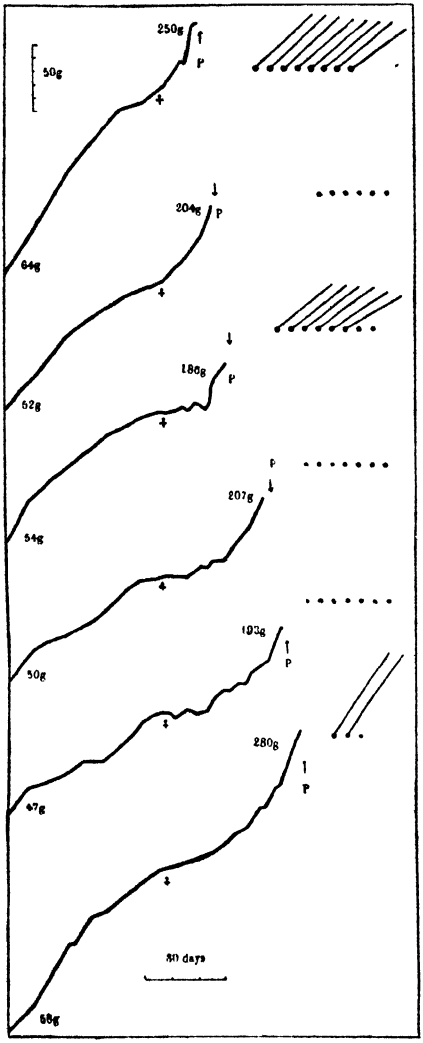

Chart 1—Growth curves of 6 females of the test series supplied with liver extract, showing the details of breeding experiment. Three of the females suckled their young.

The Growth of the Young.

The growth of the young suckled by the mother rats fed with liver extract was remarkably good.

In Chart 3 we represented all the young belongining to three litters by a series of vertical columns, the body weight on the 21st day after birth being indicated by the height of the column. The last column, which is shaded to distinguish it from tne others, represents the standard average body weight of rats at the age of 22 days, given in Donaldson's tables.(2)

An inspection of this chart is sufficient to show that the growth of suckling rats in our own series is excellent, so much so that the poorest in growth among them at the age of 21 days is equal in weight to Donaldson's average standard for 22 days old rats. The great majority of our young rats exceeded by far this average standard.

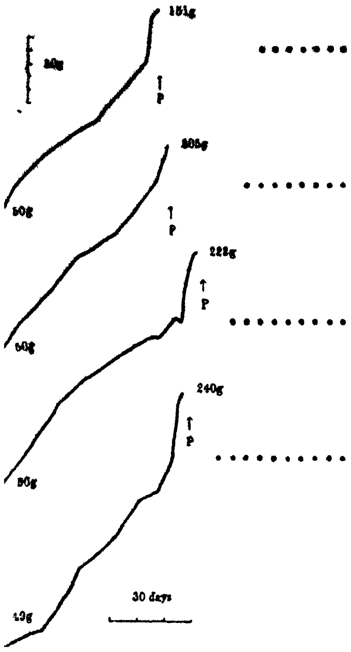

Chart 2—Growth curves of 4 control females, not supplied with liver extract. Young were not suckled by any of these females.

The opening of the eyes of the young took place on about the fourteenth day after birth, which is quite normal.

Comparison of Mammary Glands.

There remains to be described the functional state of the mammary glands of the females which failed to suckle their young in the above experiment.

We have already referred to the fact that in the females supplied with liver axtract, the mammary glands showed evidence of active milk secretion. A gross anatomical examination was sufficient to reveal this evidence. The glandular tissue was well developed and the numerous ducts and tubules of the glands were very clearly marked by their milk contents so that the entire gland gave the appearance of being thickly invested with milky network.

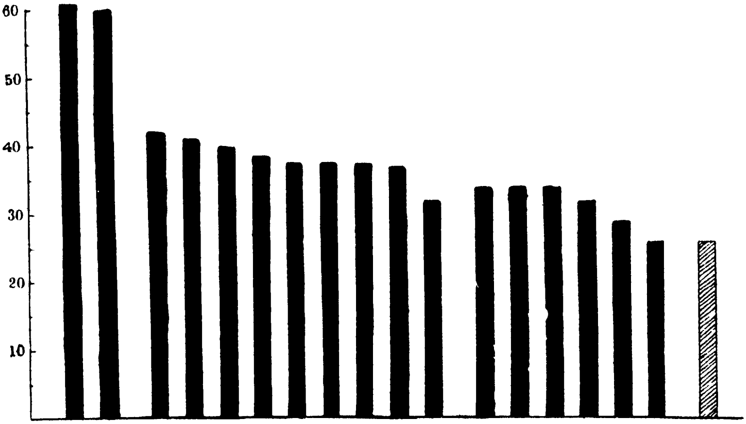

Chart 3 —Body weight at 21 days of age of our liver extract series (solid), compared with Donaldson's average standard for 22 days old rats (shaded).

These gross appearances were confirmed by histological examination (Figure 1). Here the interlobular adipose tissue is reduced to the minimum through the expansion of glandular lobules, and the alveoli composing the lobules are all actively secreting.

A striking contrast to the above condition was offered by the general appearance of the mammary glands in rats failing to lactate on our basal diet. The glandular tissue was poorly developed and the ducts and tubules of the glands were either not clearly visible in gross or were only partially detectable by their scanty milk contents.

Histologically, the gland contained much adipose tissue in which were seen relatively small glandular lobules. Most of the alveoli were inactive, alveoli were seen here and there. See Figure 2.

These findings definitely establish that even though these two sets of rats failed to suckle the young, there is no such inhibition of milk secretion in the group supplied with liver extract as constantly occur in controls fed on the basal diet alone.

Comments.

The foregoing data prove that beef liver contains a substance which is capable of strikingly promoting the process of lactation, but does not necessarily demonstrate that this lactation promoting substance is different from vitamin B, especially vitamin B_2. However, the fact that liver is rich in vitamin B_2, which is held by certain authors to be important for lactation, dose not seem to us sufficient to explain our results. Our basal diet which

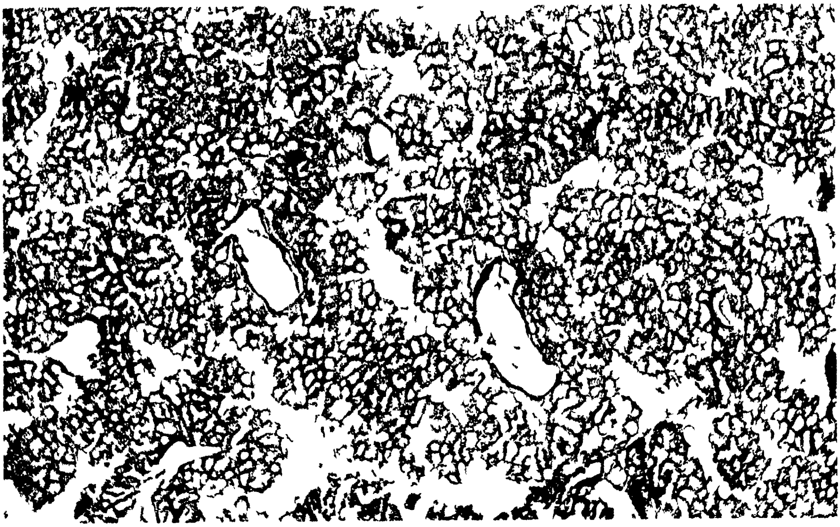

Figure 1—From a rat supplied with liver extract showing the expansion of glandular lobules composed of actively secreting alveoli

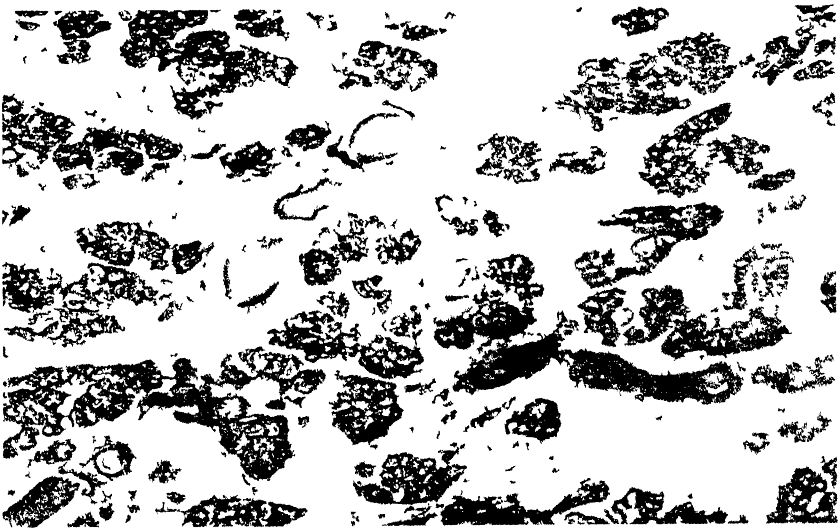

Figure 2—From a rat maintained on basal diet alone Same magnification as Figure 1

Photomicrographs of mammary glands (inguinal) of rats failing to suckle the young killed 3 days after parturition is inadequate for successful lactation, contains yeast in the high ratio of 5%, so that the additional vitamin B_2 in liver extract can hardly be expected to bring about any radical improvement in the diet.

It may be suspected that liver contains a new dietary principle allied to but not identical with vitamin B_2, and that this factor plays an important rolè in the process of lactation. Whether or not this supposed lactation promoting principle is identical with Mapson's(3) growth promoting "physin" is not determinable at this moment.

Conclusion.

Addition of liver extract to a well balanced synthetic diet which is inadequate for lactation, renders this diet largely capable of supporting active lactation, thus demonstrating the existence in liver of a dietary principle which has a distinct lactation promoting action.

We take this opportunity of expressing our cordial thanks to Professor U. Suzuki, M. I. A., for his kindly interest and helpful criticisms.

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(1) W. Nakahara and F. Inukai: Sc. Pap I. P. C. R., **22**, 301, (1933).
(2) H. H. Donaldson: The Rat, Philadelphia, (1924).
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On the Occurrence of Vitamin B2 (=Vitamin G) in Sake and Sake-Kasu (Pressed Cake).

By

Fumito INUKAI, Tuneto HIGASI and Waro NAKAHARA.

[Reprinted from Sci. Pap. I. P. C. R., Vol. 24, pp. 113—118, 1934]

The recent increasing interest in vitamin B_2 (or vitamin G) lead us to investigate the possible occurrence of this important vitamin in some characteristically Japanese fermentation products, and as the first step we tested sake (rice wine) and sake-kasu.

A few years ago Takahashi and Rin(1) published their experiments showing the occurrence of both vitamin B_1 and B_2 in sake-kasu. It appears from this paper, however, that they used potato starch in their basal diet. Potato starch is now known to contain traces of growth promoting substance and therefore to be unsuitable as a constituent of a basal diet for vitamin B_2 tests. We ourselves observed a fairly good growth of albino rats on a diet composed of potato starch 75 g, purified fish protein 10 g, butter 10 g, McCollum's salt mixture 5 g and oryzanin 4 c.c.[3]

In the following experiments we used rice starch, tested and proved to be deficient in vitamin B complex, as the source of carbohydrate in basal diet in order to obtain reliable information. We also made use of the adsorption by acid earth as a convenient method of concentrating vitamin B complex, and while acid earth adsorbate may not be commendable for use in human subjects, we found it quite satisfactory for the purpose of animal experiments.

Experiments.

Growth Promoting Action of Sake-Kasu.

Young albino rats of approximately 50 grams body weight were divided into two groups, and one group was maintaind on the following basal diet

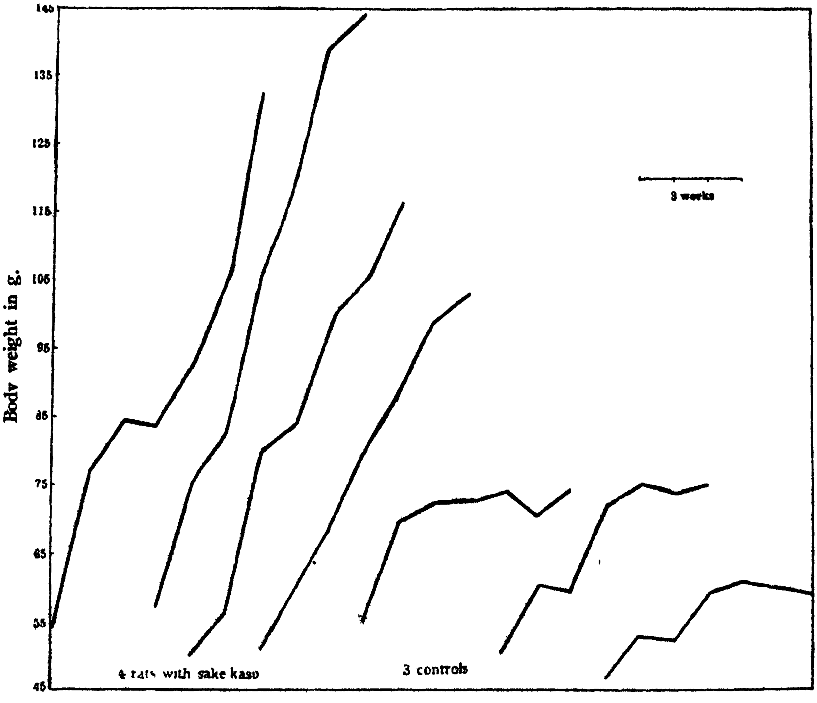


Chart 1.—Growth curves of rats on vitamin B_2 deficient diet with and without sake-kasu supplement.

which is free from vitamin B_2 :—

Polished rice powder	75 g
Fish protein	10 g
Butter	10 g
McCollum's salt mixture	5 g
Oryzanin	4 c.c.

The other group was fed on the same diet as above but with the addition of 8 g (dry weight) of sake-kasu per 100 g of the basal diet.

The result of this experiment, as shown in Chart 1, clearly indicates a marked growth promoting action of sake-kasu.

Adsorption of Vitamin B_2 in Sake-Kasu by Acid Earth.

In view of the well established fact that vitamin B_2 as well as vitamin B_1 are easily and completely adsorbed by a number of adsorbents, we carried out additional tests using acid earth adsorbate of the extracts of sake-kasu.

Sake-kasu was extracted with twice the volume of 60% methyl alcohol containing a small amount of sulphuric acid. To 1 litre of filtered clear extract was added 20 g of white acid earth, and was kept at the temperature of 30°C for 20 minutes with occasional stirring. The acid earth was recovered by filtration and was washed with water, with alcohol, and then with ether. Forty grams of the acid earth so treated was added to 100 g of the same vitamin B_2 deficient diet as used in the preceding experiment.

Since the acid earth adsorbate of sake-kasu extract should carry with it all the available vitamin B_1 along with vitamin B_2, tests were also made for the possible existence of vitamin B_1. The vitamin B_1 deficient basal diet used was the same as the vitamin B_2 deficient diet except that oryzanin was omitted from the composition.

Examples represented in Chart 2 suffice to prove that acid earth adsorbate of sake-kasu is a very potent source of vitamin B_2, but not of antineuritic vitamin B_1.

In Chart 2, Rat No. 1 was maintained on the vitamin B_2 deficient diet containing vitamin B_1 (oryzanin) for three weeks, after which acid earth adsorbate was incorporated into this diet. The typical growth response of the rat following the adsorbate feeding demonstrates the presence in the adsorbate of vitamin B_2.

Rat No. 2 was maintained on the same diet but without the oryzanin supplement, i. e., the diet deficient in both vitamin B_1 and B_2. The addition of the adsorbate to the diet failed to induce growth, and the rat died of typical polyneuritis. This shows that the adsorbate dose not contain antineuritic vitamin B_1.

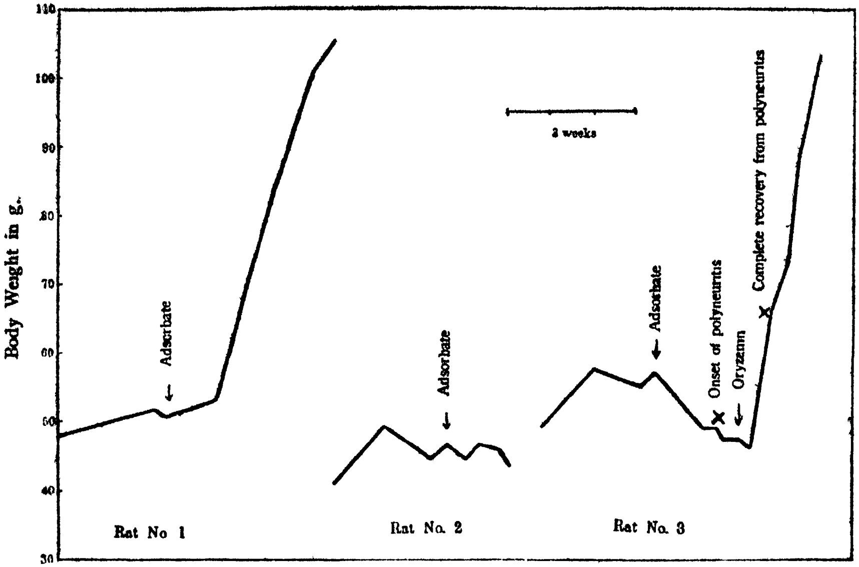


Chart 2.—Vitamin B_1 and B_2 tests of acid earth adsorbate of sake-kasu extract

Rat No. 3 was fed on the same diet as Rat No. 2, both vitamins B_1 and B_2 being absent. As in the case of Rat No. 2, addition to the diet of the adsorbate called forth no growth response, and the rat manifested typical symptoms of polyneuritis. Oryzanin was, therefore, incorporated into the diet in addition to the adsorbate, upon which the rat recovered rapidly from polyneuritis and at the same time showed very active growth. This demonstrates again that the adsorbate is deficient in vitamin B_1 but is rich in vitamin B_2.

Presence of Vitamin B_2 in Raw Sake.

The presence of vitamin B_2 in sake-kasu naturally leads to the supposition that sake itself may also contain this vitamin.

One litre of sake was acidified by adding 10 c.c. of sulphuric acid and then treated with 20 g of white acid earth, and the adsorbate was separated in the same manner as in the preceding experiment.

Acid earth adsorbate was prepared of a certain popular brand of bottled sake on the market. Tests were performed using the same vitamin B_2 deficient diet as before, and they proved that this adsorbate had no growth promoting action (Chart 3, Rats Nos. 1～3). Forty grams of adsorbate was added to 100 g of basel diet in these teste.

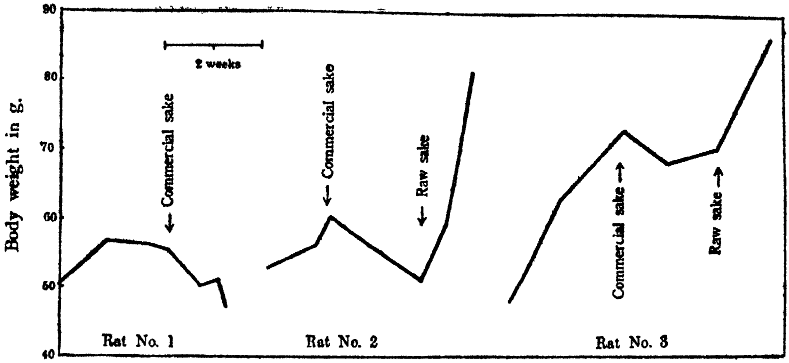


Chart 3.—Vitamin B_2 tests of acid earth adsorbate of raw and commercial samples of sake.

Acid earth adsorbate was prepared also of raw sake ("Ozeki" brewery of Nada) before it was heated to stop further fermentation (the processs called "hiire" among sake brewers) preparatory to placing it on the market. This adsorbate, added in the amount of 40 g per 100 g of basal diet, brought about typical growth respones, indicating the presence of vitamin B_2 (Chart 2, Rats Nos. 2 and 3).

This striking difference between the bottled sake on the market and the raw before the 'hiire" treatment, is probably due to the fact that sake producers treat sake with animal charcoal in order to decolorize the commercial products, with the resultant removal of growth promoting vitamin.

Comments.

The growth promoting vitamin in sake and pressed cake is without doubt derived from sake yeast. It may therefore be of interest to note that 8 g (dry weight) of sake-kasu is sufficient to serve as an adequate source of vitamin B_2. Since only about half the bulk of sake residue is composed of yeast, this amount of sake-kasu may be regarded as representing 4 g of yeast.

We know through our experience that under similar experimental conditions the amount of beer yeast required as an adequate source of vitamin B_2 may be 3 to 5 g. While it is difficult to arrive at a definite conclusion without making accurate quantitative comparison, it would seem safe to asume that sake yeast may not be inferior to beer yeast as to the vitamin B_2 content.

From the point of view of human nutrition it is of interest that sake-kasu, which is often consumed in Japan as an accessory food is now proved to be a rich source of vitamin B_2. As regards the vitamin B_2 content of sake itself, attention should be called to the fact that a bottled sample of a popular brand of sake on the market was found to be devoid of vitamin B_2, probably due to the adsorption method of decolorization to which commercial sake is subjected preparatory to distribution.

Conclusion.

Animal experiments described in this paper demonstrate that sake-kasu and raw sake are good sources of growth promoting vitamin B_2.

Antineuritic vitamin B_1, however, does not seem to be contained in these fermentation products.

The foregoing experiments were conducted under the supervision of Professor U. Suzuki, to whom we desire to express our appreciation. Our thanks are also due to Mr. Nagashima of the Ozeki Brewery, Nada, for his kindness in donating a sample of raw sake.

Literature.

(1) E. Takahashi and K. Rin. J. Agr. Chem. Soc. Japan, 5, 177. (1929).
(2) Unpublished observation.

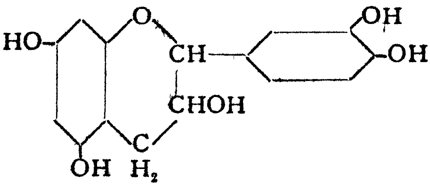
Isolation of a New Catechin, Tea Catechin II or Gallo-Catechin from Green Tea.

By

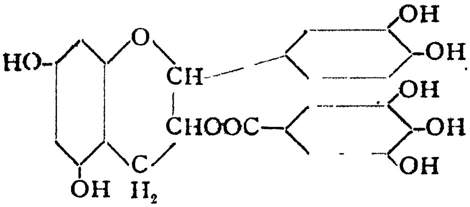
Michiyo TSUJIMURA

(Received May 15, 1934.)

A few years ago, the author has isolated tea catechin(1) and tea tannin(2), (3) from green tea and assigned the following formulae to them.



Tea catechin $C_{15}H_{14}O_6$



Tea tannin $C_{22}H_{18}O_{10}$

Quite recently, a new catechin having the formula $C_{15}H_{14}O_7$ m p 218° has been isolated in crystalline state (Photo. 1) and the name Tea catechin II or Gallo-catechin has been proposed by the author in order to distinguish it from the first catechin, which shall be designated now as Tea catechin I.

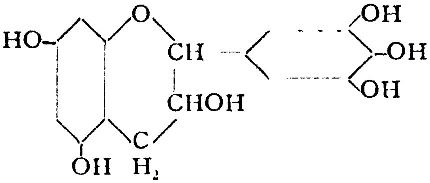
The acetyl derivative of the new catechin forms rhombic crystals (Photo. 2) m p 189~190° and corresponds to the formula $C_{15}H_8O{\cdot}(OCOCH_3)_6$. The methyl derivative (Photo. 3) obtained from the acetylation product is represented by the formula $C_{15}H_8O(OH){\cdot}(OCH_3)_5$ m p 183°.

The acetyl derivative gives phloroglucinol when boiled with concentrated alkali and the methyl derivative forms trimethyl gallic acid by cautiously oxidizing with $KMnO_4$.

The specific rotation of the new catechin is $[\alpha]_D^{18} = -67.5°$ and closely resembles to that of Tea catechin I i. e. $[\alpha]_D^{25} = -69°$.

The absorption spectrum (Photo. 4) is exactly the same with that of the methyl derivative (Photo. 5) but differs somewhat from that of Tea catechin I (Photo. 6).

From these observations, the following formula has been assigned to the new catechin.



Tea catechin II $C_{15}H_{14}O_7$
(Gallo-catechin)

A catechin existing in Malett and Pistacia is presumed by K. Freudenberg(4) to have the same formula. Further, a new tannin $C_{15}H_9O_2(OH)_5$? isolated by Y. Oshima and co-worker(5) from the fresh tea leaves of Formosa seems to be identical with the new catechin of the present author. These points will be investigated later on.

Experimental.

1. *Isolation of New Catechin from Green Tea.*

The material used in this experiment was the green tea of superior quality, prepared in Shizuoka prefecture. It was extracted three times with water at ordinary temperature, and the extract was treated with neutral lead acetate solution until no more precipitate was formed. The greenish precipitate thus produced, was collected, washed with water, and decomposed with 10% sulphuric acid. The reddish yellow filtrate from the lead sulphate was now repeatedly extracted with ethyl acetate, and the extract was evaporated under reduced pressure while passing a current of carbon dioxide. The residue was then dissolved in a small volume of water and after shaking with ether three times, the aqueous layer was separated and evaporated to expel off the ether, and filtered. On keeping the filtrate in an ice box, needle-shaped crystals separated out, which were washed with water, dried on a porous tile and extracted with chloroform in Soxhlet apparatus. The insoluble residue was then dissolved in hot water and filtered while hot. On cooling the filtrate in an ice box, the new catechin separated out: m p 218° (uncorr.). The yield was about 0.25% of the material used.

From inferior green tea no crystals were obtained by the same treatment.

2. *Chief Properties of Tea Catechin II.*

The freshly prepared Tea catechin II is colourless needles, soluble in water, alcohol, ethyl acetate and aceton; difficultly soluble in ether, and insoluble in chloroform and benzen. It is gradually oxidized in the air. The aqueous solution has an astringent taste with sweetish after-taste, and gives violet colour with ferric chloride. It gives also the characteristic phloroglucinol reaction with pine shaving, moistend with concentrated hydrochloric acid, but it gives no precipitate with gelatin. When heated with dilute acid it is converted into a reddish brown substance.

For comparison, the chief reactions of Tea catechin II, Tea catechin I and Tea tannin are given in the following table:

	Tea catechin II	Tea catechin I	Tea tannin
Astringency	weak	weak	strong
Aqueous solution treated with			
$FeCl_3$	violet	green	blue
Gelatin	no ppt	no ppt	white ppt
heated with			
dil acid	reddish brown ppt	reddish brown ppt	reddish brown ppt and gallic acid
Phloroglucinol reaction	positive	positive	positive

3. *Analysis of Tea Catechin II.*

	Sample (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
I.	3.547	7.452	1.584	57.30	5.00
II.	3.628	7.638	1.568	57.42	4.84
III.	3.454	7.303	1.496	57.67	4.85
IV.	3.462	7.321	1.384	57.67	4.74
	Calc. for $C_{15}H_{14}O_7$			58.81	4.61

4. *Specific Rotation of Tea Catechin II.*

$$[\alpha]_D = \frac{\alpha \times 100}{c \times l}$$

α: observed rotation	-0.675
c: concentration	1% in 95% ethyl alcohol
l: length of tube in decimeter	1

$$[\alpha]_D^{18} = -\frac{0.675 \times 100}{1 \times 1} = -67.5^\circ$$

5. *Acetyl Derivative.*

Two grams Tea catechin II were dissolved in 10 c.c. acetic acid anhydride, cooled and treated with one drop of concentrated sulphuric acid, the reaction started at once. After several hours the reaction mixture was poured into water and the precipitate thus formed was collected after standing for several hours, washed with water and dried on a porous tile. For purification it was dissolved in hot methyl alcohol and filtered while hot. On cooling the filtrate the acetyl derivative separated as rhombic crystals (Photo. 2), m p 189~190° (uncorr.).

6. *Analysis of the Acetyl Derivative.*

	Sample (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
I.	3.709	7.878	1.513	57.93	4.56
II.	3.218	6.827	1.367	57.86	4.75
	Calc. for $C_{15}H_8O(OCOCH_3)_6$			58.04	4.70

7. *Molecular Weight of the Acetyl Derivative.*

The determination was carried out by Rast's camphor method.⁽⁶⁾

$$m = \frac{c \times w \times 100}{dW}$$

m: mol wt.	
c: const.	100
w: subst. mg	16.1
d: depress. of m p	5°
W: solvent mg	225

$$m = \frac{400 \times 16.1 \times 100}{5 \times 225} = 572$$

Calc. for $C_{15}H_8O(OCOCH_3)_6$ 558

8. *Methyl Derivative.*

The methyl derivative of Tea catechin II was prepared from the acetylation product described above.

Two grams of the latter were dissolved in 10 c.c. methyl alcohol, to which 15 c.c. dimethyl sulphate were added and treated with 15 c.c. 50% KOH drop by drop with shaking. The reaction product was poured into water, and after standing for some hours the precipitate was collected, washed with water and dried on a porous tile. For purification it was dissolved in hot methyl alcohol and filtered while hot. On cooling, the methylated product crystallized out (Photo. 3), m p 183° (uncorr.).

9. *Analysis of the Methyl Derivative.*

	Sample (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
I.	3 126	7.285	1.830	63.56	6.55
II.	3.121	7.260	1 775	63 44	6.36
	Calc. for $C_{15}H_8O(OH)(OCH_3)_5$			63.80	6.43

10. *Decomposition of the Acetyl Derivative with Concentrated Potash.*

The acetylated product was boiled with 50% KOH at 180° for 30 minutes. After cooling, the reaction mixture was diluted with water, neutralized with sulphuric acid, filtered and sufficient sodium bicarbonate was added until weakly alkaline, again filtered and extracted with ether. The etherial extract was evaporated and treated with water. From the aqueous solution phloroglucinol separated out in crystalline state; m p 210°.

It gave the characteristic reactions of phloroglucinol; mixed with pure phloroglucinol no depression of melting point was observed.

11. *Oxidation of the Methyl Derivative with Potassium Permanganate.*

The methyl derivative was dissolved in methyl alcohol, heated on a water bath and a strong solution of potassium permanganate was added drop by drop until the red colour remained unchanged on standing, the excess of permanganate was then decomposed with dilute sulphuric acid until slightly acid and extracted with ether. The etherial extract was evaported and the crystalline residue thus obtained was treated with sodium bicarbonate to remove the phenolic substance and recrystallized from hot water. It was identified as trimethyl gallic acid; colourless needles, m p 168°, mixed with pure specimen no depression of melting point was observed.

Analysis :–

	Sample (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
I.	3 251	6.740	1.690	56.54	5.82
II.	3 124	6 495	1.640	56.70	5.87
	Calc. for trimethyl gallic acid				
	$(CH_3O)_3C_6H_2CO_2H$			56.58	5.70

12. Absorption Spectra.

The absorption spectra of Tea catechin II, its methyl derivative, Tea catechin I, and of Tea tannin are shown in Photos. 4, 5, 6 and 7. (Spectrograph, Hilger's Quartz type; source of illumination, Hydrogen Geisler tube.)

The author expresses her sincere thanks to Prof. U. Suzuki for his kind direction and encouragement throughout this work, and also thanks to Mr. Funahashi for helping on the elementary analysis.

Literature.

(1) Tsujimura Bull. Agric. Chem. Soc. Japan. **6**, 62, (1930).
(2) 〃 〃 **6**, 70, (1930).
(3) 〃 〃 **7**, 23, (1931)
(4) K. Freudenberg Tannin Cellulose, Lignin, 66, (1933).
(5) Y. Oshima J. Agr. Chem. Soc. Japan, **9**, 948, (1933)
(6) Karl Rast Ber., **55**, 1051, (1922).

Photo. 1—Tea catechin II from aq. sol. 1 : 100

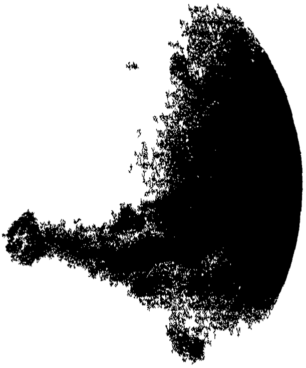


Photo 2—Acetyl derivative of Tea catechin II from methyl alc sol 1 100

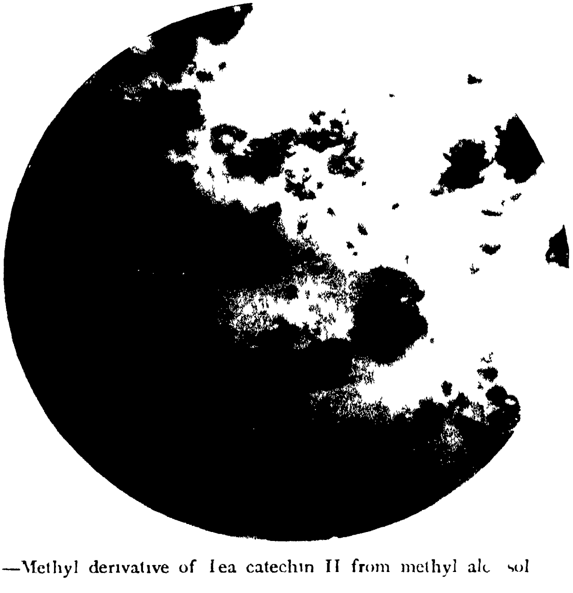


Photo 3—Methyl derivative of Tea catechin II from methyl alc sol 1 100

Photo 4—Tea catechin II, $\frac{1}{1,000}$ mol in alcohol

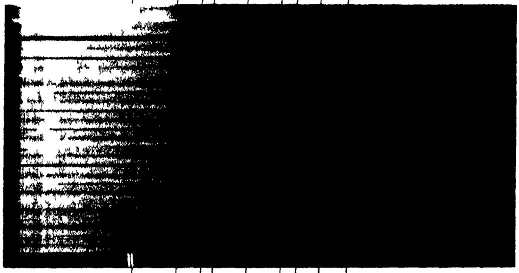


Photo 5—Methyl derivative of Tea catechin II $\frac{1}{1,000}$ mol in alcohol

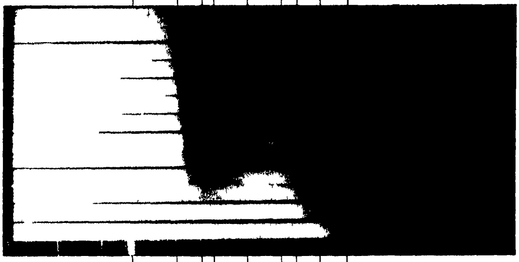


Photo 6—Tea catechin I $\frac{1}{1,000}$ mol in alcohol

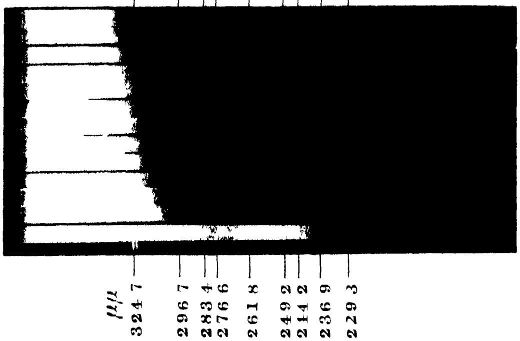


Photo 7—Tea tannin $\frac{1}{1,000}$ mol in alcohol

Über die Hygroskopicität der Düngemitteln.

Von

Matsuo TOKUOKA.

(*Wissenschaftl. und landw. Abteilung, Taihoku Universität, Taiwan*).

(Eingegangen am 10. August 1934)

Es wurde die Hygroskopicitat von 20 Arten der Handelsdünger durch die statische Methode verglichen. Die zum Versuche benutzen Düngemitteln sind folgende:

Harnstoff, Harnstoffnitrat, Leunasalpeter I. G., Phosphazote, Ammophos I, Ammophos II, Diammophos I. G., Kalksalpeter I. G., Ammonsulfat I. G., Kalkstickstoff, Ammoniumnitrat, Kalisalpeter, Chilesalpeter, Vit'azote, Sojabohnenkuchen, Superphoshat, Rohphosphat, Rapskuchen, Kalisulfat und Kalichlorid.

Die Temperature und die Feuchtigkeit, denen die Dungemitteln ausgesetzt wurden, wurden aus den Mittelwerten der niederschlagereichsten Monaten in Tokio (1921～1925), namlich Juni, Juli, August und September, berechnet. Die mittlere Temperatur war 23,5°C, und die mittlere relative Feuchtigkeit war 81,4%. Bei jedem Versuche war die Temperature immer 23,5°C, aber die relative Feuchtigkeit wurde geandert, d. h. 70%, 75%, 81,5 %, 85%, 90% und 95%.

Der gewünschte Wasserdampfdruck wurde mittels des Gemisches von Wasser und Schwefelsaure hergestellt. Die Hygroskopicitat wurde durch die Gewichtzunahme der in dem Glaschen gelegten Probe, die in dem Behalter 10 Tage über dem Gemisch von Wasser und Schwefelsaure gehalten wurde, ermittelt. Kalksalpeter, Vit'azote, Phosphazote, Ammoniumnitrat und Leunasalpeter haben sehr starke Hygoskopicitat gezeigt. Dagegen haben Kalisalpeter, Harnstoffnitrat, Ammonsulfat und Kalisulfat sehr schwache Hygroscopitat gezeigt. Die andere Düngemittel liegen zwischen diesen zwei Gruppen betreffs des Hygroskopicitat.

Further Evidence for the Occurrence of Vitamin E in Soy Bean Oil.

By

Umetaro SUZUKI, Waro NAKAHARA
and Yoshikazu SAHASHI.

(Reprinted from Sci. Pap I P C. R. Vol. 24, No. 517, pp 283∼286.)

In a previous paper⁽¹⁾ we presented a preliminary report on the occurrence of vitamin E in soy bean oil. The evidence for this conclusion available at that time consisted of the fact that soy bean oil incorporated in a liberal amount to an otherwise vitamin E deficient diet prevented the degenaration of testicles, while other oils, such as peanut oil, cocoanut oil, white sesame oil, palm oil, or blubber oil of finback whale, had no such protective effect on the male germ cells as shown by soy bean oil. Although we were fully aware that the testicular degeneration could not of itself be regarded as a positive proof of vitamin E deficiency, the composition of the diet and the excellent physical conditions of the rats were such that we did not hesitate to conclude that our series of rats fed on oils other than soy bean oil were suffering vitamin E deficiency.

The proof of the existence of vitamin E, however, is incomplete without breeding tests, and it is the purpose of this paper to describe the results of our subsequent experiment which supplies this needed evidence.

Experiment

The synthetic diet used was of the following composition :-

Potato starch (J P)	65 g
Fish protein	15 g
Soy bean oil	10 g
McCollum's salt mixture	5 g
Dried yeast	5 g

Biosterol (in olive oil), 1 mg per rat daily, separate from the above mixture.

Fish proteins and yeast were thoroughly extracted with ether. We did not subject potato starch to ether extraction, since, from the result of our previous experiment, we know that potato starch does not contain enough vitamin E, if any, to interfere with the object of our test.

The soy bean oil used was a guaranteed pure product supplied by the Nisshin Seiyu Co.

The experiment was started with 7 pairs (males and females) of young albino rats, which were fed on the above diet throughout the entire experimental period of 170 days. In the course of the experiment 3 of the males died of intercurrent infection, and we were forced to allot 2 females to a single male in some cases.

Results

The outcome of the experiment is presented in the accompanying chart (Chart 1) in the form of growth curves with annotations.

Female No. 1 became pregnant toward the 50th day after the beginning of the experiment, but it resulted in abortion. About 30 days later the female

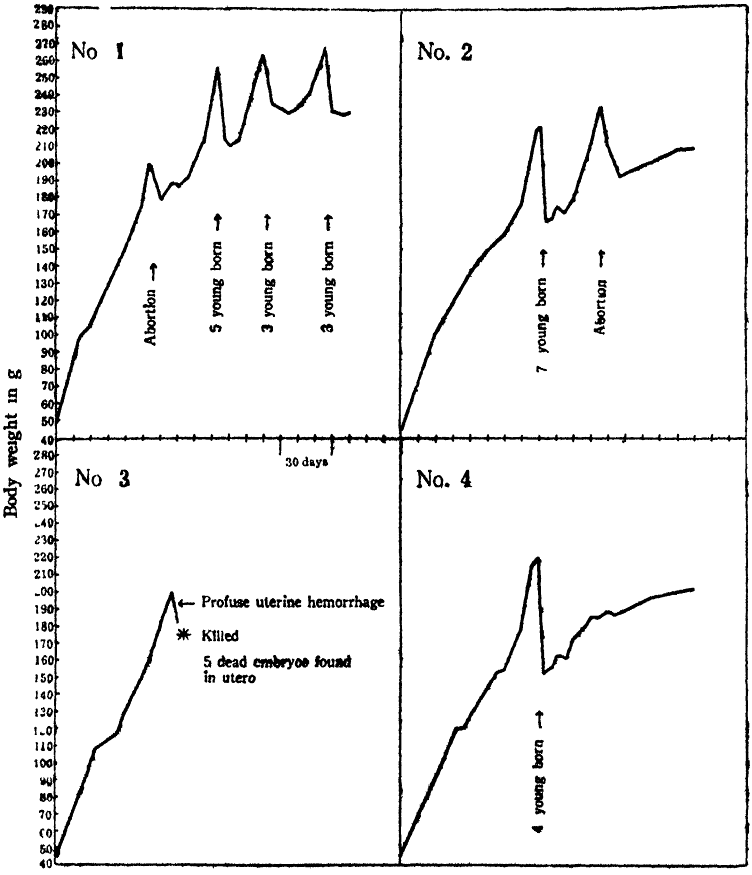


Chart 1 —Growth curves and reproductive incidences of the 4 female rats referred to in the text.

again became pregnant and this time gave birth to 5 young. Some 30 days later it again gave birth to 3 young. The female became pregnant the fourth time and produced 3 young. On all the occasions the young died within two or three days after birth through the failure of lactation on the part of the mother rat.

Female No. 2 gave birth to 7 young 83 days after the beginning of the experiment. The young did not survive due to the failure of lactation. A second pregnancy took place but this resulted in abortion.

Female No. 3 became pregnant about 50 days after the commencement of the experiment. A severe disturbance developed, however, toward the last period of gestation and on the 70th day the animal was found dying from a profuse hemorrhage from the uterus, and was therefore killed. Autopsy revealed 5 fairly large embryos *in utero*, but these were already dead and were undergoing partial disintegration. This is an undoubted case of the interruption of gestation.

Female No. 4 gave birth to 4 young on the 81st day, but the young did not survive. This rat did not become pregnant again for the rest of the experimental period.

Females No. 5, No. 6 and No. 7 did not become pregnant throughout the entire course of experiment. We attach no special significance to these cases since it is quite usual in breeding experiments to find a certain proportion of females that will fail to become pregnant. Growth curves of these females need not be given here.

Four males used for mating were killed at the termination of the experiment, i.e., after 170 days, and autopsies performed. Not only were these rats in excellent physical condition, but they also showed testicles absolutely normal without any sign of degenaration. The testicles weighed 1.1～1.2 g.

Conclusion

The result of the breeding test above described confirmed the conclusion at which we had previously arrived, namely, that soy bean oil contains antisterility vitamin E. We found that female rats were usually able to go through with normal gestation and give birth to litters on a synthetic diet with soy bean oil as the only source of vitamin E. However, to judge from the fact that interruption of gestation occurred on a few occasions, the amount of vitamin E contained in soy bean oil may be relatively small.

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(1) U. Suzuki, W. Nakahara and Y. Sahashi: Sc. Pap. I. P. C. R., **23**, 270, (1934).

Chemical Studies on "Bukuryo", Sclerotia of *Pachyma Hoelen* RUMPH. (VI).

Hydrolytic Oxidation Products of *β-Pachyman* by Conc. Sulphuric Acid and Conc. Nitric Acid.

By

Kenzi TAKEDA.

(Received August 6, 1934.)

β-Pachyman was treated with a mixture of conc. H_2SO_4, conc. HNO_3 and water at low temperature, which produced no nitro pachyman when the mixing rate of acids and water attained a certain limit as follows, for each 2 g β-pachyman:

H_2O	H_2SO_4 (1.840) (g)	Fum. HNO_3 (1.52) (g)	Temp	Time (hr)	Yield of nitrate (g)
6.3	12.9	14.1	17~19°	24	0
7.5	12.4	13.4	〃	〃	0
8.4	11.9	12.9	〃	〃	0
9.5	11.4	12.3	〃	〃	0
8.4	11.9	12.9	10~12°	〃	0.0181

On the contrary, it seems highly probable that oxygluconic acid and glucuronic acid were found to be in these aqueous solutions, as oxydised compounds produced by the complex hydrolytic oxidation, in addition to glucose. They could not have been purely isolated, but two portions separated showed satisfactory positive results enough to identify the presence of the above two compounds.

The Characteristics of the Lactic Acid Bacteria Isolated from Moto, Yeast Mashes for Sake Manufacture.

Part. I. The Fermentation Products From Koji Extract.

By

Hideo KATAGIRI and Kakuo KITAHARA.

(Received August 10, 1934.)

The 39 strains of lactic acid bacteria were isolated in the year of 1933 from 38 kinds of moto and in 1934, 18 strains were isolated from 16 kinds of moto and a sample of koji, by plate cultures on chalked maltose yeast water agar.

The 57 strains thus obtained, were classified into two species according to their forms: 37 strains were found to be streptococcus (0.7 μ) and the others were short bacilli ($0.8 \times 1.5 \sim 3.0\ \mu$).

It will be seen in Table I that all the short bacilli produced almost theoretically lactic acid from koji extract, while koji extract was decomposed into not only lactic acid but also alcohol and CO_2 by the streptococci, without any exception, therefore the manner of decomposition of glucose was as follows:

by short bacilli····· $C_6H_{12}O_6 = 2C_3H_6O_3$··········· ·· · ····· (1)

by streptococci·· ·· $C_6H_{12}O_6 = C_3H_6O_3 + C_2H_6O + CO_2$······ (2)

Table I. Fermentation products from koji extract.

Lactic acid bacteria	Average yield of products (%)				Number of strains producing				
	lactic acid	volatile acid	alcohol	CO_2	*l*	*d*	*dl*	*l*+*dl*	*d*+*dl* lactic acid
short bacillus (20 strains)	101.4	2.3	0.7	0	0	7	7	0	6
streptococcus (37 strains)	47.3	2.4	21.7	23.3	36	0	0	1	0
calculated from equation (1)	100.0	0	0	0	—	—	—	—	—
〃 〃 〃 (2)	50.0	0	25.6	24.4	—	—	—	—	—

It is interesting to be pointed out that not only the manner of decomposition of glucose but also the optical rotation of lactic acid produced from koji extract was found to be different between the short bacilli and the streptococci. Table I shows that all the streptococci produced totally *l*-lactic acid excepting only one strain with which the lactic acid consisting of 70% of *l*- and 30% of *dl*-forms was obtained, while *l*-lactic acid was never found in the products with the short bacilli, since *d*- or *dl*- lactic acid were obtained with each 7 strains and the other 6 strains produced the acid consisting of the above two forms.

The Characteristics of the Lactic Acid Bacteria Isolated from Moto, Yeast Mashes for Saké Manufacture.

Part II. Observation of Fermentable Sugars and Some Cultural Conditions.

By

Hideo KATAGIRI, Kakuo KITAHARA and Kanji FUKAMI.

(Received August 10, 1934)

All the 57 strains of lactic acid bacteria isolated from moto (see Part I) produced acid form pentose, sucrose and glucose, while acid production was hardly detected from glycerol, inulin and mannitol. It will be seen in Table I that arabinose, fructose and maltose were rapidly fermented by almost all the bacteria, galactose was also fermented by most of them although the degree of production of acid was inferior to the formers Rhamnose, starch, dextrin and lactose were fermented by least number of strains, while fairly large number of bacteria produced acid from α-methyl glucoside and salicin.

It will be interesting to note that the fermentability of xylose was remarkably different between the streptococci and the short bacilli, since it was fermented rapidly by all the cocci, while the bacilli were found generally to have no fermenting power of xylose with the exception of three strains by which slight formation of acid was pointed out.

Table I. Kinds of fermentable sugars.

Lactic acid bacteria	Number of strains producing acid from															
	xylose	alabinose	rhamnose	glucose	fructose	galactose	sucrose	maltose	lactose	dextrin	starch	inulin	α-methyl glucoside	salicin	glycerol	mannitol
Streptococcus (37 strains)	37	36	0	37	37	34	37	37	2	1	0	0	25	15	0	0
Short bacillus (20 strains)	3	20	3*	20	19	16	20	18	5	4	4*	0*	4*	5*	0	1

* represents the results of experiments on which 14 strains were under examination.

The maximum acid production was again found to be different between the streptococci with which pH value of fermented koji extract was reached to 3.5 and the short bacilli with which pH=4.0 was observed to be the average value.

The optimum pH value for the multiplication was found to be about 6.0 with all the bacteria, but a remarkable difference in the resistance to alkali was found between streptococci and short bacilli; almost all the former strains grew well on a medium of pH=10.5, while any growth of the latter strains was never observed on such alkaline solution.

It was observed with all the strains that the optimum and limiting temperatures were 30° and about 40° respectively and 35% glucose or 5% alcohol inhibited greatly the multiplication of these bacteria, although their growth was taken place on such media containing 25% glucose or 3% alcohol.

The Characteristics of the Lactic Acid Bacteria Isolated from Moto, Yeast Mashes for Saké Manufacture.

Part III. The Fermentation Products from Pentoses and Hexoses.

By

Hideo KATAGIRI, Kakuo KITAHARA, Kanji FUKAMI
and Minami SUGASE.

(Received August 10, 1934.)

It is already pointed out (see Part I) that the manner of decomposition of glucose was divided into two classes: all the short bacilli (20 strains) produced lactic acid from glucose according to the equation (1), while glucose was decomposed by all the streptococci (37 strains) into lactic acid, alcohol and CO_2 as was shown in the equation (2).

$$C_6H_{12}O_6 = 2C_3H_6O_3 \qquad (1)$$

$$C_6H_{12}O_6 = C_3H_6O_3 + C_2H_5OH + CO_2 \qquad (2)$$

Any differece was not found between the fermentation products from arabinose by the short bacilli and those of arabinose and xylose by the streptococci, therefore it is concluded all the bacteria decomposed pentoses according to the equation (3).

$$C_5H_{10}O_5 = C_3H_6O_3 + CH_3COOH \qquad (3)$$

The fermentation of galactose or mannose was observed to be taken place in the same manner as was pointed out with glucose. Thus the equation (1) and (2) were found to be applicable not only to glucose but also to all the aldohexoses, for the short bacilli and the streptococci respectively.

The short bacilli were again found to decompose fructose following to the equation (1) however, almost all the streptococci produced mannitol from fructose excepting two strains by which fermentation of fructose was taken place according to the equation (2).

It was suggested that alcohol was directly produced from fructose by the mannitic streptococci by which mannitol was hardly fermented, therefore the new equation (5) which was composed of equations (4) and (2) was proposed for the fermentation of fructose by the streptococci.

$$3C_6H_{12}O_6+H_2O=2C_6H_{14}O_6+C_3H_6O_3+CH_3COOH+CO_2 \cdots\cdots\cdots (4)$$

$$28C_6H_{12}O_6+3H_2O=18C_6H_{14}O_6+10C_3H_6O_3+9CH_3COOH+C_2H_5OH+10CO_2 \cdots\cdots (5)$$

The Characteristics of the Lactic Acid Bacteria Isolated from Moto, Yeast Mashes for Saké Manufacture.

Part IV. The Classification of the Lactic Acid Bacteria.

By

Hideo KATAGIRI, Kakuo KITAHARA and Kanji FUKAMI.

(Received August 10, 1934.)

All the 57 strains of the bacteria isolated from moto were found to be true lactic acid bacteria according to their morphological characters, physiological nature of not reducing nitrate etc.

It was pointed out that the bacteria were divided into two species: 20 strains of short bacilli and 37 strains of streptococci, since remarkable differences were found between them not only in their microscopical appearance but also in the manner of fermentation of aldohexoses and fructose, optical rotation of the lactic acid being produced, fermentability of xylose, the maximum acid production, the resistance to alkali and in the reduction of litmus.

When the streptococci were compared with Leuconostoc mesenteroides, Hucker and Pederson, very much the same natures were found, between them as was shown in Table I, therefore Leuconostoc mesenteroides var. Saké was proposed to represent these strains,

It was concluded that the short bacilli were classified as a new species as Lactobacillus Saké, although nearly the same characteristics were found in Streptobacterium plantarum, Orla-Jensen and Lactobacillus arabinosus, Fred, Peterson and Anderson (see Table I) with which remarkable differences were still pointed out in their fermentability of lactose, salicin and mannitol, coagulation of milk, reduction of litmus etc.

Table I. The characteristics of the lactic acid bacteria.
(% of the total strains).

Lactic acid bacteria		Streptococcus	Leuconostoc mesenteroides	Short bacillus	Sbm plantarum	Lactob. arabinosus
Number of strains		37	97	20	9	3
Size (μ)		0.7	0.6-0.9	0.8×1.5-3.0	0.8-1.0× 3.0-8.0	0.5-0.6× 1.2-2.0
Optimum temp. (C)		30	21-30	30	30	30
Acid production from	xylose	100	70	15	20	0
	arabinose	97	87	100	100	100
	rhamnose	0	15	21*	33	67 (slowly)
	glucose	100	96	100	100	100
	fructose	100	98	95	100	100
	galactose	92	97	80	100	100
	sucrose	100	100	100	100	100
	maltose	100	98	90	100	100
	lactose	5	78	25	100	100
	dextrin	3	18	20	89 (slowly)	—
	inulin	0	rarely	0*	33	—
	starch	0	rarely	29*	0	—
	salicin	41	74	36*	100	100
	α-methyl glucoside	68	—	29*	—	0
	mannitol	10	generally	5	100	100
	glycerol	0	rarely	0	44 (slowly)	0
Formation of	CO_2 & alcohol	100	100	0	0	0
	mannitol	95	generally	0	0	0
	slime	100	generally	0	0	0
Milk coagulation		8	20	0	44	100 (softly)
Rotation of lactic acid		l=97 dl+l=3	l	d=35 dl=35 dl+d=30	dl and rarely d	dl

* represents the results of experiments on which 14 strains were under examination.

On the Natural Pigments of Raw Silk Fibre of the Domestic Cocoon (Part VII).

On the Pigments of the Green Cocoon (Bombyx mori, var. Seihaku).

By

Masami Oku.

(From the Chemical Laboratory of Gunze Raw Silk Mfg. Co. Ltd., Ayabe-mati, Kyôto-hu, Japan)

(Received August 22, 1934.)

The natural coloring matters of the greenish domestic cocoon (Bombyx mori, var. Seihaku) had hitherto been investigated by several authers, but no one had carried out the complete identification upon the chemical entity of the pigments. I have investigated to isolate the pigments in their purest state and gained new compounds, named by the auther as "Bombycin" (glucoside) and "Bombycetin" (aglucone of bombycin), which were characteristic to have each one atom of nitrogen and show many reactions and absorption bands like flavone derivatives. There were another flavone-like pigments in small quantity which were ether soluble and that found in the mother liquor of bombycin, but the auther could not precisely study them owing to the shortness of material.

Bombycin is a monoglucoside of bombycetin which has a molecular formula near as $C_{20}H_{19}O_7N$ and has three hydroxyl groups. Bombycetin shows two absorption bands of which maximum are near 2700 and 3750 $1/\lambda$ and shows flavone-like reaction. Acetyl bombycetin was obtained through acetylation by acetic anhydride and sodium acetate. It has a molecular formula near as $C_{26}H_{25}O_{10}N$ and has three acetyl groups. Its absorption band shows horizontal portion between 3100 and 3600 $1/\lambda$ and differs utterly from that of flavone or flavonol acetate.

The following items are discussed.

(1) The origin of bombycin can be asked in the mulberry leaves but it must be synthesized biochemically in the body of silk worm (Bombyx mori, var, Seihaku) owing to the fact that the auther fried negative to isolate the nitrogenous flavone-like substances from the leaves. The presence of nitrogenous flavone-like pigments, bombycin and bombycetin, in animal products is, indeed, very noticeable and interesting fact, because no one has yet found flavone-like substances from the animal origin except from the butterfly (Melanargia galatea).

(2) The physiological function of the presence of bombycin in the cocoon layers is perhaps to protect the pupa from the injurious action of the ultraviolet ray.

(3) Bombycin and bombycetin are with bombilupeol deemed as characteristic components found in the green cocoon (Bombyx mori, var. Seihaku).

Experimental.

The yellowish green cocoon layers were extracted by cold 70% alcohol several times and the extract was concentrated and divided into two fractions, ether soluble and water soluble. From the former fraction bombilupeol and ether soluble pigment were searched, to the latter fraction was added neutral lead acetate and the orange yellow precipitates formed were digested with dilute hydrochloric acid. Crude bombycin was precipitated by neutralizing the filtrate by sodium hydroxide. As crude bombycin could not be obtained in any crystal form, it was purified by dissolving in dilute hydrochloric acid and reprecipitated by neutralization. Bombycin is a monoglucoside of bombycetin and melts and decomposes over 300°C and shows many analogous character with flavone-glucosides except that it contains nitrogen. It is brownish yellow powder and shows some imbibitional properties.

Bombycin was hydrolysed by 5% H_2SO_4 and its aglucone, bombycetin, was obtained. From mother liquor of bombycetin, glucose was detected as glucosazone.

As bombycetin could not be obtained in any crystal form by any method, it was first converted into its acetyl derivative and then regenerated by saponification.

Acetyl bombycetin is a slightly yellowish powder which melts between 135～142°C and has a molecular formula near as $C_{20}H_{18}O_4(CH_3CO)_3N$. By inspection of its absorption bands, it has a characteristic feature to have absorption horizontal between 3100～3600 $1/\lambda$ (in alcohol) and differs utterly from that of flavone or flavonol acetates. It is easily soluble in glacial acetic, alcohol and acetic ether; insoluble in water, alkalies and acids. Color reaction by $FeCl_3$ is negative but reduction color by Mg+HCl is after a long time positive (red).

Bombycetin was obtained as follows: after boiling acetyl bombycetin in the mixed solution of glacial acetic acid and few drops of sulphuric acid, the mixture was poured into cold water and the precipitates formed were recrystallized from 50% alcohol. It could not be obtained in crystal form and was yellowish brown amorphous powder. It shows following reaction.

Reduction color by Mg+HCl············intense red.

$FeCl_3$……………………dark green～dark brown.
Lead acetate ………………orange red precipitates.

It is easily soluble in alcohol, aceton and pyridin but insoluble in ether, water and dil. HCl and changes brownish red by touching to alkalies. It melts between 170～180°C and at the same time decomposes by foaming. It has a molecular formula near as $C_{20}H_{19}O_7N$ and has three hydroxyl groups. It could not be methylated easily by dimethyl sulphate and the decomposition substances by alkali fusion could not be obtained in sufficient quantity to identify. It has two absorption maximum 2700 and 3750 $1/\lambda$ (in alcohol) which resemble that of quercetin.

Bombycetin is a flavone-like pigment and yet has nitrogen. That this nitrogen cannot be deemed as mere impurity but as a element of bombycetin is sure from the fact that it is estimated as one atom from both acetyl bombycetin and the regenerated bombycetin.

Ether soluble pigment was obtained from mother liquor of bombilupeol which was hitherto obtained by the auther and published in this bulletin. The pigment has many analogous properties with bombycetin.

Mother liquor of bombycin was concentrated and after adding alcohol, the filtrate was condensed to syrupy and hydrolysed by 5% H_2SO_4. After repeating the extraction with ether, ether solution was evaporated and the residue was dissolved in alcohol. From that solution, brownish yellow crystal appeared in needles in small quantity which showed many analogous properties with bombycetin especially in respect to the absorption bands.

Chemical Studies on "Bukuryo", Sclerotia of *Pachyma Hoelen* RUMPH. (VII).

The Production of Tetracetyl Glucuronic Acid by Acetolysis of *β-Pachyman* in the Presence of Perchloric Acid.

By

Kenzi TAKEDA.

(Received September 6, 1934.)

In completely acetylated pachyman ($C_2H_4O_2$, about 14%) obtained in the previous study (I) was treated with acetic acid, acetic anhydride and the small amount of perchloric acid, and acetolysis was carried out by controling the temperature and the time of heating, till the solution became clear, and at which time the contents were taken out and treated in the usual way.

(1) Acetate which was insoluble in hot water but soluble in cold 50% methanol gave a crystalline white powder, having action on Fehling's solution, M. P., 83~86°. Acetic acid, 71.69%. Specific rotatory power, $[\alpha]_D^{20}$ + 9.56° in chloroform and +8.47° in benzene. Molecular weight found was 504 in benzene by the cryoscopic method. This seemed to be a bionic acid or a mixture of acetyl biose and acetyl glucuronic acid.

(2) Acetate soluble in hot water, gave priamatic colourless crystals (Fig. 1) after recrystallization from hot 50% methanol solution, having reduction with hot Fehling's solution and with cold $KMnO_4$ solution. M. p., 103 ~107°. It contained no chlorine. Moreover, the following results were obtained with this crystal.

(a) Saponification products by 1% methanolic ammonia, gave a syrup which did not crystallize. Bial's HCl-orcin, Neuman's orcin and Tollen's naphthoresorcin reactions gave positive results of glucuronic acid.

With 1 g of syrup, phenylosazone test was carried out and no glucosa-

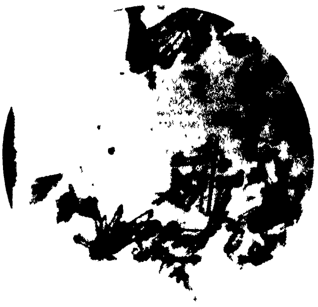


Fig. 1 (1×3)

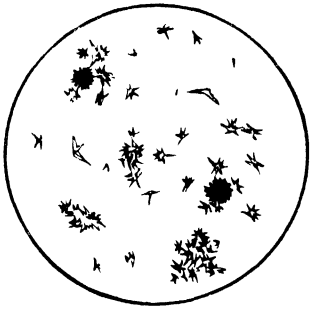


Fig. 2

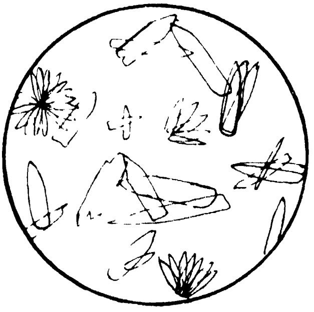


Fig. 3 (3×8)

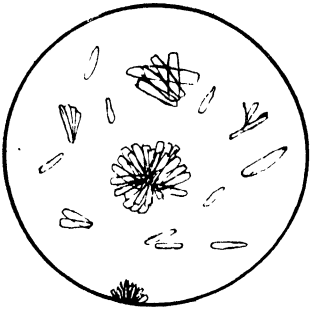


Fig. 4 (3×8)

zone was found. Osazone obtained, however, was a bur like crystal and was soluble in hot water, but with more difficulty than maltosazone. M.p. of osazone, 167∼174°. It was soluble in acetic acid, ethanol, acetone and hot water but slightly soluble in benzene and chloroform. Nitrogen content was 14.16% (required, 15.06% for $C_{18}H_{20}O_5N_4$). Molecular weight found was 320 and 345 by Rast's method (required, 372 for $C_{18}H_{20}O_5N_4$).

0.1 g syrup was hydrolysed with 2.5% HCl for 2.5 hours and the resultant syrup gave also the same osazone as above.

Osazones obtained are showed in Fig. 2, 3 and 4.

(b) Acetic acid of acetate found, 83.82% and 83.40% (required 82.87% for $C_6H_6O_7(C_2H_3O)_4$, considering all the acid radicals as acetic acid).

(c) Specific rotatory power, $[\alpha]_D^{20}$ +65.6° in benzene (C, 4.0), +66.2° in chloroform (C, 4.0) and +60.9° in methanol (C, 1.89).

(d) Molecular weight was 423, 392 and 398 in benzene by the cryoscopic method, 370 and 371 by Smith and Young's method (required, 362 for $C_6H_6O_7(C_2H_3O)_4$).

(e) Elementary analysis found was C, 45.90% and 46.02%, H, 5.28% and 5.29% (required, C, 46.40%, H, 5.00% for $C_6H_6O_7(C_2H_3O)_4$).

(f) Acetate was readily soluble in benzene, chloroform, ethylacetate, methanol, acetone and ether, soluble in carbon tetrachloride, acetic acid, toluol and pyridine, almost insoluble in carbon bisulphide and insoluble in water.

From certain of the above figures, one can consider that this may be identical with an acetate which may be accepted as tetracetyl glucuronic acid, though it has never before been reported to have been obtained.

Polysaccharide (VIII).

Ein neues Kohlenhydrat „Xyloglukronid" aus Kadsura japonica Dun.

Von

Kitsuji NISHIDA, Hideo HASHIMA und Tokuichi FUKAMI.

(*Holzchemisches Institut, Kyushu Kaiserliche Universität, Fukuoka, Japan.*)

(Eingegangen am 6, September 1934)

Kadsura japonica ist über Südjapan und Ostindien verbreitet. Der korkrindige Stamm liefert in kaltem Wasser einen gallertartigen Schleimstoff,

aus dem durch Alkohol ein hellbraun gefarbter, voluminöser Niederschlag ausgefällt wird. Der so erhaltene Schleimstoff (2 gr) wurde mit 300～400 ccm Wasser übergossen, darauf wurde er im Autoklav erhitzt und 3 Stunden bei 120～125°C gehalten. Das durchgelaufene Filtrat wurde mit Tierkohle entfärbt. So wurde ein klares, ungefarbtes Filtrat erhalten. Zu diesem wurde nun unter Umrühren ein gleiches Volum $CuSO_4$ Lösung hinzugefügt. Man gewinnt dann eine schon blau gefarbte, voluminöse Fällung, aber sie wird nicht, wie Glukomannan, Xylan, usw. durch Fehlingsche Lösung ausgefällt. Mit alkoholischer Salzsaure zerlegt man diese Aufschlämmung, filtriert das erzeugte Produkt und wäscht es mit Alkohol und Äther. Nach Abgiessen des Äthers und Trocknen im Vakuum auf Phosphorsäureanhydrid ergab sich ein schneeweisses, lockeres Pulver, das mit Jod keine Blaufärbung mehr zeigt und Fehlingsche Lösung nicht mehr reduziert.

Ausbeute und Aschengehalt waren wie folgt:

Holzteil des korkrindigen Stammes	41%
Hellbraun gefärbter Niederschlag, der durch Alkohol ausgefällt wird	0,17%
Schneeweisses Pulver, das durch $CuSO_4$ Lösung ausgefällt wird	0,07%

Wassergehalt (%)	Aschengehalt (%)
8,34	0,10
7,94	0,26
8,00	0,24
8,03	0,22

$[\alpha]_D^{20} = +49{,}0^\circ$ in 5% Wasser.

$[\alpha]_D^{20} = +45{,}8^\circ$ in 5% Wasser nach 24 Stunden.

$[\alpha]_D^{20} = +50{,}6^\circ$ in 5% Wasser mit 2 Tropfen Salzsäure nach 1 Stunde.

Die Makroelementaranalyse ergab:

	Gefunden: %		Berechnet für: $(C_{11}H_{16}O_{10})_n$ %
C	42,26	42,79	42,84
H	6,09	6.10	5,32

Der Xylangehalt wurde nach der Phloroglucinmethode von Tollens und der Kohlensauregehalt nach dem Lefèvre Verfahren bestimmt und ergab folgende Analysewerte:

	Gefunden: %				Berechnet für $(C_{11}H_{16}O_{10})_n$ %
Kohlensäure	13,16	13,11	12,52	12,72	14,28
Glukuron	52,62	52,43	50,07	50,89	57,12
Xylan	41,45	——	41,53	41,37	42,86

Aus diesen Versuchen geht also hervor, dass das weisse Pulver aus 1-Mol. Xylan und 1-Mol. Glukuron aufgebaut ist. Hier wurden, um zahlenmässige Schlussfolgerungen aus den Angaben herzuleiten, die Fälle berücksichtigt, bei denen zu diesem Zwecke Hydrolyse versucht wurde, und wobei

gleichzeitig der Nachweis der Xylose, Glukuronsäure und anderer Kohlenhydrate ausgeführt wurde. Diese Untersuchungen werden zusammen mit denen über Acetylierung, über Methylierung und ausserdem über die Konstitution in einer späteren Mitteilung genauer erklärt werden.

On the Natural Pigments of Raw Silk Fibre of the Domestic Cocoon (Part VIII).

Isoquercitrin from Mulberry Leaves.

By

Masami Oku.

(From the Chemical Laboratory of Gunze Raw Silk Mfg. Co, Ltd., Ayabe-mati, Kyôto-hu, Japan.)

(Received September 10, 1934.)

The author had already studied the natural pigments of Japanese green cocoon (var. Seihaku) and isolated from it a new pigment, "Bombycin", which showed flavonol-glucoside reaction and contained one atom of nitrogen.

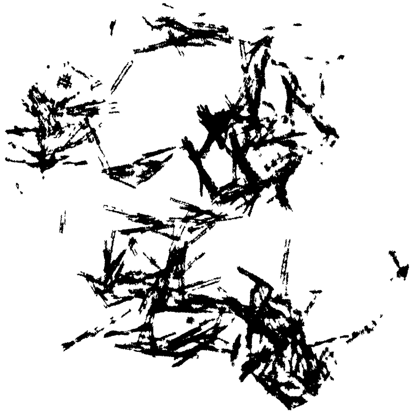
The author tried to isolate bombycin from mulberry leaves but could never find it and, in place of it, isolated fine crystallizable compound, which was identified as isoquercitrin, which is a quercetin monoglucoside (3-glucoside).

Isoquercitrin was already isolated from brown-husked maize by Sando and Bartlett and from cotton flower by Perkin, but the author was the first to isolate it from mulberry leaves.

Preparation of Isoquercitrin.

The air dried ground powder of mulberry leaves was extracted with boiling 80% alcohol three times and the extract was concentrated, which was shaken with ether and ether soluble substances were rejected. To the water soluble fraction was added neutral lead acetate and the yellow precipitate formed was decomposed by hydrogen sulphide. Seeing that both the lead sulphide and its filtrate contained considerable amount of flavonol glucoside, it was removed by repeated shakings with ethyl acetate. The ethyl acetate extracts were then evaporated and the residue was dissolved in adequate amount of water and then shaken with toluene and left still in a ice box for several days. The crude glucoside was separated and purified by repeated crystallization from boiling water, finally by dilute aqueous pyridine. Thus about 3.2 g of pure glucoside was obtained starting from 7100 g of air dried leaves (0.05% yield). The glucoside, in its final state of purity, forms pale yellow needle-shaped plates as shown in Fig. 1.

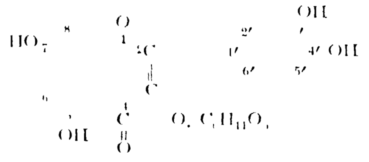
Fig. 1



Isoquercitrin, of mulberry leaves crystallized from dilute aqueous pyridine (×380).

Identification of Isoquercitrin.

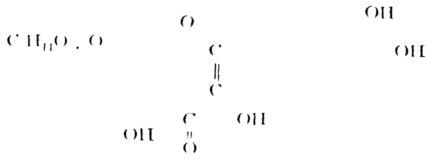
The pure glucoside melts at 220～221° (corr.) and has no water of crystallization and upon hydrolysis by 5% sulphuric acid resolved into quercetin and glucose in molecular proportions. There are two quercetin monoglucosides identified till to-day, cited as follows,



Isoquercitrin

(M. P. 217～219° by Perkin
220～222° by Sando)

Quercetin-3-glucoside



Quercimeritrin

(M. P. 247～249°)

Quercetin-7-glucoside

The author has identified the glucoside as 3-glucoside by methylation and by its absorption spectrum as follows. The pure glucoside was methylated two times by diazomethane ether solution and gained pale yellow needles separated which melted at 150～152°. This methyl glucoside was hydrolysed by 1.5% sulphuric acid and slightly yellow needles separated, which by recrystallization from benzene melted at 196～197°. These results coincided utterly with the data given by Attree and Perkin (J. C. S. 1927, 234), who had proved isoquercitrin to have glucose in 3-position of quercetin.

By inspection of the absorption spectrum of the glucoside and its aglucone quercetin, I have obtained the following results from their absorption curves.

Absorption maxima (in frequency)

	glucoside	aglucone
First Band	2850	2700
Second Band	3800	3800

(Concentration: 1/10,000 mol alcohol solution)

These data, too, showed the position of glucose in 3 of quercetin molecule.

Sterilizing Power of Fruits Juice and Condiments on Bac. Typhosus and Vib. Cholerae.

Sogo TETSUMOTO.

(Received September 10, 1934.)

I. Materials.........Fruits juice, Condiments, Strong mineral acid.
II. Experiment. (1) Resisting power of used microorganisms. (2) Experimental method. (3) Detection of test bacteria.
III. Determination of pH of test materials.
IV. Results.
V. Summary.

According to the strong sterilizing action of acids, by Krönig u. Paul's(1), H. Shimokawa's(2) and auther's(3) etc. were reported previously. Fruits, condiments and veverages which are eaten usually raw are almost acid.

I studied on the sterilizing action of fruits juice, condiments and veverages for Bac. typhosus and Vib. cholerae and compared the strength of sterilizing power of many materials for prophylactic purpose.

I. Materials.

I choose such 37 materials as those we like and always use at daily life, avoiding to choose the superior quality.

I.	Fruits juice	16 kinds.
II.	Seasonings	19 kinds.
III.	Strong mineral acids	2 kinds.

Name of material chosen and chief components of sour taste or sterilizing substances are as shown in the following table.

I. Fruits.

	Name of materials		Component of sour taste
Rutaceae	Mandarin orange.	Citrus Aurantium, L. nobiles Makino.	Citric acid
	Chinese citron.	〃 〃 , L. subsp. amara, Engl.	〃
	Washington Navel orange.	〃 sinensis. Osbeck.	〃
	Lemon.	〃 medica, L. subsp. Limonum Hook	〃
	Yuzu (fresh).	〃 Aurantium, L. subsp. Junos. Makino	Citric acid, *l*-malic acid
	Yuzu (shop-worn)	〃 〃 〃 〃	〃
Rosaceae	Apple (sour).	Mallus pumilla, Mill. var. domestica	*l*-malic acid, Citric acid
	Apple (not so sour)	〃 〃	〃
	Pear	Pirus sinensis, Lindl. var. Culta, Makino	*l*-malic acid
	Japanese medlar.	Eriobotrya japonica Lindl.	〃
	Peach (Tientsin).	Prunus persica, L. Stokes-var. Tientsin.	*l*-malic acid, Citric acid
	Peach.	〃 〃 Stokes.	〃
	Sweet cherry.	Prunus avium, L.	〃
	Strawberry.	Strawberry variety "Victoria"	*d*-tartaric acid *l*-malic, Citric acid
Vitaceae	Black grape.	Vitis Labrusca vinefera, vit. Camp.	*l*-malic acid, *d*-tartaric acid
	Grape (Koshyu).	Vitis vinefera, L. var. Koshyu.	〃

II. Condiments and Strong mineral acids.

	Name of materials		Component of sterilization
Cruciferae	"Wasabi" (fresh and bitter)	Eutrema Wasabi japonica	Allylmustard oil
	〃 (fresh and ordinally bitter)	〃	C_3H_5CNS
	〃 (shop worn and not so bitter)	〃	〃
	Raddish (bitter)	Raphanus sativus, L.	C_3H_6CNS
	〃 (ordinally bitter)	〃	〃
Zingiberaceae	Ginger (fresh and bitter)	Zingiber officinale Roscoe	Shogaol
	〃 (ordinally bitter)	〃	Zingerone
	〃 (shop worn and not so bitter)	〃	
	"Umeboshi" pickled plum (large)		Citric acid, *l*-malic acid
	〃 〃 (small)		NaCl etc,

Sauce	A. imported	Acetic acid, Citric acid
	B. saled at "M"-departmet	Cinnamic acid, lemonen
	C. 〃 market	Pepper, ginger etc.
Soy	A. made by "Kikkoman" Co.	Formic acid, propionic acid, *d*-lactic acid, succinic acid.
	B. 〃 "Yamasa" Co.	Na-caprinate etc
	C. saled at market	
Vinegar	A. made from saké	Acetic acid.
	B. made by "Riken"	
	C. saled at market	
Strong mineral acids,	results of HNO_3, HCl and H_2SO_4 were the same, so I denote the results of HNO_3 and H_2SO_4	*N*/1000 and *N*/10000 of HNO_3 and H_2SO_4
Control	Ringer's solution	

II. Experiment.

(1) Resisting power of used microorganism.

Degree of resisting power of microorganisms used has great influence on the strength of germicidal activity of materials(1). I choose next 4 kinds of infectious microorganisms having constant resisting power for phenol aqueous solution referened on G. Reddish's method(4) and standard methods of examining disinfectants. Among Bac. typhosus and Vib. cholerae, I' and II' have the very strong resisting power for phenol aqueous solution. On prophylactic purpose I choiced such strong resisting bacteria especially and compared the result of standard test bacteria.

Surviving period (minute)	Phenol aqueous solution			
	I Bac. typhosus (Standard)	I' Bac. typhosus (Strong)	II Vib. cholerae (Standard)	II' Vib. cholerae (Strong)
	90 times by weight	90 times by weight	175 times by weight	175 times by weight
5	+	+	+	+
10	+	+	±	+
15	−	+	−	+
30	−	+	−	+
45	−	±	−	−
60	−	−	−	−

+…… …alive −.. ……perished ± .. ….sometimes alive and sometimes perished

(2) Experimental method.

We washed fruits and vegetables with sterilized water and dried them at room temperature.

We gathered their juice as cleanly as possible. Juice of "yuzu" was taken by pressing its peel and fruit together.

As the Juice of "umeboshi" (pickled plum) is colloidal state, I dilute it by adding 2 times by volume of sterilized water. Taking 10 c.c. from each material into test tubes and kept them in an incubator at 20°C (within ± 0.5°C). Each of standard bouillon culture of the infectious bacteria was also kept at 20°C. Put 1 c.c. of bouillon culture into 10 c.c. of material and keep them at 20°C. At the certain interval I inoculated microorganisms with the certain platinum wire into standard bouillon of pH 7.0 for Bac. typhosus, and pH 8.0 for Vib. cholerae and I cultivated them 2~3 days at 37°C. Adding 1 c.c. of bouillon culture into 10 c.c. of material, seems too much the bouillon culture comparing to the material, but on prophylactical stand point of view, results of the such case will be more reasonable than the results of small quantity.

(3) Detection of test bacteria from other bacteria.

To detect the bacteria from other, I used many Endo's dish culture for Bac. typhosus and Aronson's dish culture for Vib. cholerae. Colonies of above organisms have been witnessed by morphological investigations and by hanging drop method using 1 : 100 dilution of the agglutination test with immune serum of rabbit of each microorganism. When the experiment comes near the end, and activity of microorganisms becomes faint, living force of test bacteria is suppressed by other bacteria, then the determination of alive or death is very trouble.

Though Endo and Aronson media are special one for Bac. typhosus and Vib. cholerae respectively, but these media prevent the growth of these above microorganisms in some degree.

On this respect I used many neutral agar dish cultures besides of Endo's dish culture for Bac. typhosus, and alkali agar dish culture besides Aronson's dish culture for Vib. cholerae.

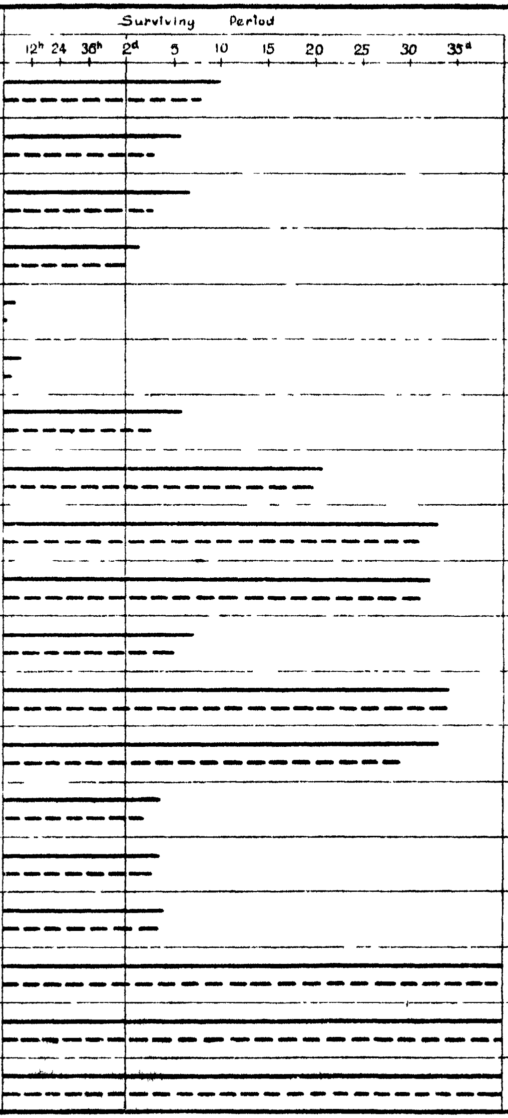
III. Determination of pH of test materials.

Each juice of fruits, "wasabi", ginger, or "umeboshi" (pickled plum) differs their pH respectively.

I mixed juice of the same kind of material which appears in the market generally. I determind pH by colorimetric method.

At vinegar, its original state, at Riken vinegar and strawberry juice I diluted 10 times by volume. At soy and sauce 50 times and others are all 5 times I diluted and determined pH of these materials.

Table I. Sterilizing power of various Fruits juice on Bac. typhosus.



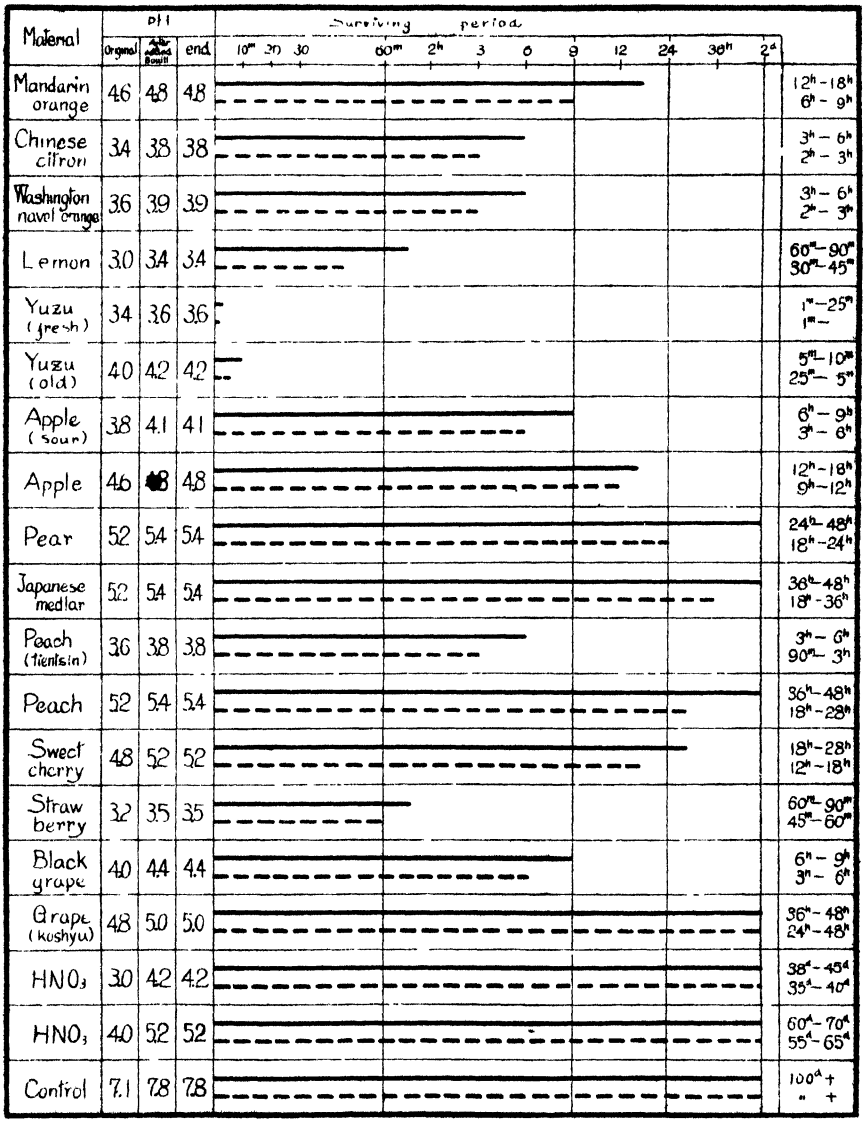
Material	pH Original	pH After adding Bacillus	pH end	Surviving Period	
Mandarin orange	4.6	4.8	4.8	8^d-10^d 7^d-8^d	On 9 day fermentation bigins
Chinese citron	3.4	3.6	3.6	4^d-6^d 2^d-4^d	
Washington navel orange	3.6	3.8	3.8	6^d-7^d 4^d-5^d	
Lemon	3.0	3.3	3.3	2^d-3^d 36^h-2^d	
Yuzu (Fresh)	3.4	3.6	3.6	2^h-3^h 90^m-2^h	
Yuzu (Old)	4.0	4.2	4.2	6^h-8^h 3^h-6^h	
Apple (Sour)	3.8	4.0	3.9	4^d-6^d 3^d-4^d	
Apple	4.6	4.7	4.4	19^d-21^d 19^d-20^d	On 19 day fermentation bigins
Pear	5.2	5.3	5.4	30^d-33^d 30^d-31^d	On 30 day fermentation bigins
Japanese medlar	5.2	5.4	5.2	30^d-32^d 30^d-31^d	On 30 day fermentation bigins
Peach (Tentsin)	3.6	3.7	3.7	5^d-7^d 3^d-5^d	
Peach	5.2	5.4	5.4	33^d-34^d 32^d-34^d	On 33 day fermentation bigins
Sweet cherry	4.8	5.0	5.0	27^d-33^d 26^d-29^d	
Straw berry	3.2	3.3	3.3	2^d-4^d 2^d-3^d	
Black grape	4.0	4.1	3.8	3^d-4^d 2^d-3^d	On 2 3 day fermentation bigins
Grape (Koshyu)	4.8	5.0	4.6	3^d-4^d $3-4^d$	On 2-3 day fermentation bigins
HNO_3	3.0	4.2	4.2	90^d	After 90 days microrg +
HNO_3	4.0	5.2	5.2	100^d	After 100 days microrg +
Control	7.1	7.1	7.1	150^d	After 150 days microrg +

———— B. typhosus having the strong resisting power.

- - - - - - - - B. typhosus having the standard resisting power.

h ········ hour d ········ day + ········ survived

Table II. Sterilizing power of various Fruits juice on Vib. cholerae.



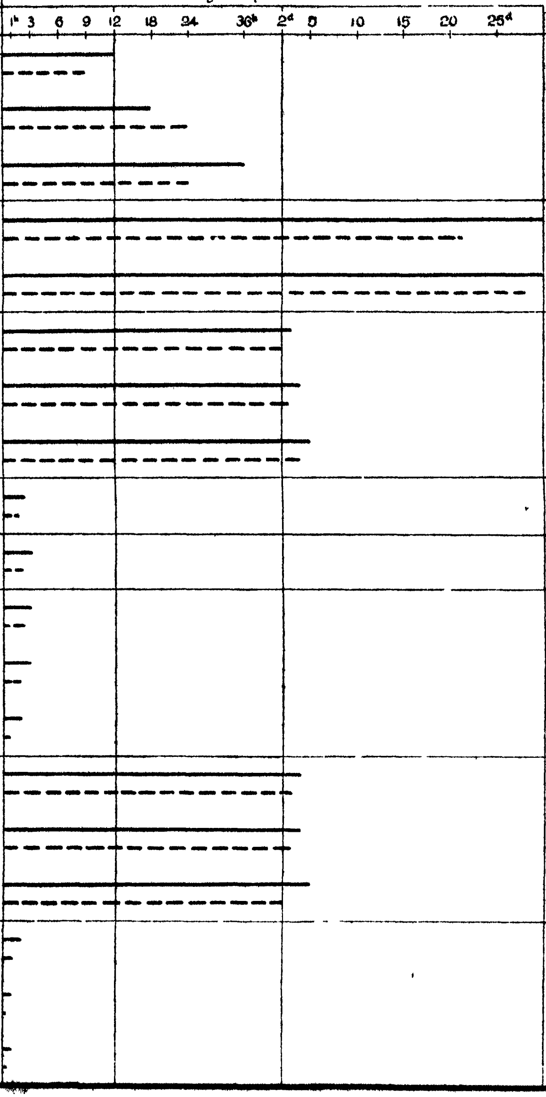
Material	pH			
	Orginal	After added Bouill	end	
Mandarin orange	4.6	4.8	4.8	$12^h - 18^h$ $6^h - 9^h$
Chinese citron	3.4	3.8	3.8	$3^h - 6^h$ $2^h - 3^h$
Washington navel orange	3.6	3.9	3.9	$3^h - 6^h$ $2^h - 3^h$
Lemon	3.0	3.4	3.4	$60^m - 90^m$ $30^m - 45^m$
Yuzu (fresh)	3.4	3.6	3.6	$1^m - 25^m$ $1^m -$
Yuzu (old)	4.0	4.2	4.2	$5^m - 10^m$ $2.5^m - 5^m$
Apple (sour)	3.8	4.1	4.1	$6^h - 9^h$ $3^h - 6^h$
Apple	4.6	4.8	4.8	$12^h - 18^h$ $9^h - 12^h$
Pear	5.2	5.4	5.4	$24^h - 48^h$ $18^h - 24^h$
Japanese medlar	5.2	5.4	5.4	$36^h - 48^h$ $18^h - 36^h$
Peach (Tientsin)	3.6	3.8	3.8	$3^h - 6^h$ $90^m - 3^h$
Peach	5.2	5.4	5.4	$36^h - 48^h$ $18^h - 28^h$
Sweet cherry	4.8	5.2	5.2	$18^h - 28^h$ $12^h - 18^h$
Straw berry	3.2	3.5	3.5	$60^m - 90^m$ $45^m - 60^m$
Black grape	4.0	4.4	4.4	$6^h - 9^h$ $3^h - 6^h$
Grape (koshyu)	4.8	5.0	5.0	$36^h - 48^h$ $24^h - 48^h$
HNO_3	3.0	4.2	4.2	$38^d - 45^d$ $35^d - 40^d$
HNO_3	4.0	5.2	5.2	$60^d - 70^d$ $55^d - 65^d$
Control	7.1	7.8	7.8	$100^d +$ " +

———— V. cholerae having the strong resisting power.

-------- Vib. cholerae having the standard resisting power.

m……minute h……hour d……day +……survived —……perished

Table III. Sterilizing power of Condiments on Bac. typhosus.



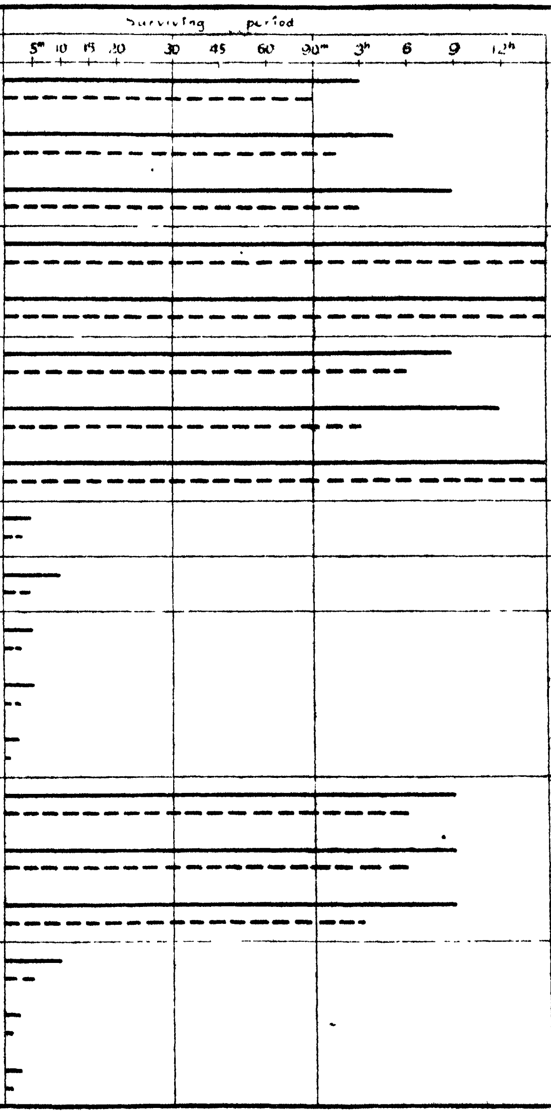
	pH original	pH after added bacillus	pH end	Surviving period
Wasabi fresh	5.0	5.2	5.2	9^h – 12^h 6^h – 9^h
" ordinal			"	12^h – 24^h 12^h – 18^h
" shopworn	5.2	5.3	5.3	24^h – 36^h 18^h – 24^h
Raddish bitter	6.0	6.6	6.6	20^d – 28^d 17^d – 21^d
" ordinal	6.2	6.8	6.8	25^d – 35^d 23^d – 28^d
Ginger fresh	5.2	5.4	5.4	2^d – 3^d 36^h – 2^d
" ordinal	"	"	"	3^d – 4^d 2^d – 3^d
" shopworn	5.3	5.4	5.4	3^d – 5^d 3^d – 4^d
Umeboshi large	3.2	3.3	3.3	90^m – 2^h 60^m – 90^m
" Small	3.3	3.4	3.4	2^h – 3^h 60^m – 2^h
Sauce A.	4.4	4.6	4.6	2^h – 3^h 1^h – 2^h
" B.	"	"	"	2^h – 3^h 90^m – 2^h
" C.	4.3	4.5	4.5	1^h – 2^h 30^m – 1^h
Soy A.	5.2	5.3	5.3	3^d – 4^d 2^d – 3^d
" B.	"	"	"	3^d – 4^d 2^d – 3^d
" C.	5.1	5.3	5.3	2^d – 5^d 36^h – 3^d
Vinegar A.	2.8	3.0	3.0	45^m – 2^h 30^m – 1^h
" B.	2.6	2.8	2.8	30^m – 45^m 20^m – 30^m
" C.	2.4	2.6	2.6	20^m – 1^h 10^m – 30^m

——————— B. typhosus having the strong resisting power.

- - - - - - - - B. typhosus having the standard resisting power.

h………hour d………day +………survived

Table IV. Sterilizing power of Condiments on Vib. cholerae.



	pH			
	Original	After added Bouillon	end	
Wasabi fresh	5.0	5.2	5.2	2^h – 3^h 1^h – 90^m
〃 ordinal	〃	〃	〃	3^h – 5^h 90^m – 2^h
shopworn	5.2	5.3	5.3	6^h – 9^h 2^h 3^h
Raddish bitter	6.0	6.6	6.6	5^d – 6^d 3^d – 4^d
〃 ordinal	6.2	6.8	6.8	6^d – 8^d 4^d – 6^d
Ginger fresh	5.2	5.5	5.5	6^h – 9^h 2^h – 6^h
〃 ordinal	〃	〃	〃	9^h – 12^h 3^h – 6^h
〃 shopworn	5.3	5.6	5.6	12^h – 18^h 9^h – 12^h
Umeboshi large	3.2	3.4	3.4	2.5^m – 5^m 1^m – 2.5^m
〃 small	3.3	3.4	3.4	5^m – 10^m 2.5^m – 5^m
Sauce A	4.4	4.6	4.6	2.5^m – 5^m 1^m – 2.5^m
〃 B	〃	〃	〃	2.5^m – 5^m 1^m – 2.5^m
〃 C	4.3	4.5	4.5	1^m – 2.5^m 1^m +
Soy A	5.2	5.4	5.4	6^h – 9^h 3^h – 6^h
〃 B	〃	〃	〃	6^h – 9^h 3^h – 6^h
〃 C	5.1	5.2	5.2	3^h – 9^h 2^h – 3^h
Vinegar A	2.8	3.0	3.0	5^m – 10^m 2.5^m – 5^m
〃 B	2.6	2.8	2.8	1^m – 2.5^m 1^m ±
〃 C	2.4	2.6	2.6	1^m – 2.5^m 1^m –

———— V. cholerae having the strong resisting power.

- - - - - - Vib. cholerae having the standard resisting power.

m…minute h…hour d…day +…survived —…perished ±…

IV. Results.

The results obtained are as described in the following table I∼IV.

From the above experiments and the results as shown in table I∼IV, we know the following facts. In general, sterilizing power of fruits juice for Bac. typhosus and vib. cholerae is strong as proportional to their strength of sourness.

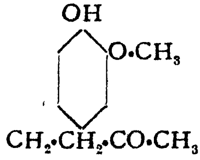
Sterilizing action of these juices due to the action of acid components such as citric acid, *l*–malic acid and *d*–tartaric acid, contained in materials.

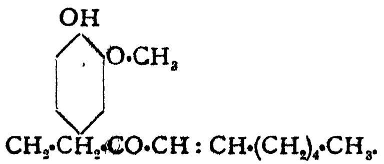
When fruits juice begin fermentation, viability of used bacteria suddenly decays and in relatively short time bacteria perishes.

Change of pH of juice at before and after about fermentation, is relatively small. Accordingly the cause of death of the bacteria is due to the fermentation product of fruits juice rather than the change of pH of the material. Strong sterilizing action of "yuzu" juice is due to the combinated action of citric acid, *l*–malic acid, glucosids and several essential oils such as *d*–limonen, citral and citronellal etc.

If we compare the sterilizing power of strong mineral acids having nearly the same pH of strong sour or not so sour fruits juice and condiments to that of used materials, we see that test materials have far stronger sterilizing power than strong mineral acids as shown in table I∼IV.

By these facts we know that the sterilizing action of fruits juice and condiments is due to the complex action of essential oils and other substances beside pH. By the results noted in table III and IV, we see next facts.

Except raddish juice, many condiments have far stronger sterilizing power than fruit juices generally. The cause of this is due to the complex components other than pH of materials. From the results of "wasabi", ginger and raddish juices, we see that sterilizing power of materials is proportional to the fresh. Among fresh, if we compare the sterilizing power about the degree of bitterness, sterilizing power is proportional to the degree of bitterness. Chief bitter component of "wasabi" and raddish juice is allyl mustard oil C_3H_5CNS and that of ginger are zingerone 

and shogaol 

Accordingly as chief sterilizing component of "wasabi" and raddish juice, we think allyl mustard oil, and of ginger, may be zingerone and shogaol.

"Umeboshi" (pickled plum) has very strong sterilizing power, its sterilizing power due to citric acid and other unknown substance. The strong sterilizing action of vinegar is chiefly due to the action of acetic acid but besides this, other unknown substance also play strong sterilizing action.

Sauce has very strong sterilizing action. Its sterilizing action due to combined action of acetic acid, citric acid and mustard oil, zingerone and shogaol contained in pepper and ginger.

Sterilizing action of soy is relatively weak. Its sterilizing action is due to the combined action of its component such as formic, propionic, *d*–lactic and succinic acids and Na–caprinate and NaCl. Judging from the result of soy, sauce and vinegar, there is almost no difference about trade mark on sterilizing power.

I express my profound thanks to Dr. Y. Tohyama and Dr. S. Kojima for their kind guidance in my work. And also express hearty thanks to Prof. Dr. K. Shibata, Prof. Dr. Y. Asami and Prof. Dr. H. Nomura for their kind report on the component of several fruits and vegetable juices and etc.

V. Summary.

I studied the sterilizing action of 16 kind of fruit juices and 19 kind of condiments on Bac. typhosus and Vib. cholerae, and the following conclusions are obtained :—

Results are as follows.

(1) "Yuzu" juice has the strongest sterilizing power among such fruits juice as lemonen, mandarin orange and strawberry juices. Juices of Japanese medlar, peach, pear and sweet cherry have weak sterilizing power.

(2) Among condiments, vinegar, umeboshi and sauce have the extraordinally strong sterilizing power.

(3) Condiments have stronger sterilizing power than fruits juice except raddish juice.

(4) On the whole sterilizing power of fruit juices is strong as proportional to their strength of sourness. Their sterilizing action is due to the action of fatty acid such as citric, *l*–malic, *d*–tartaric. Essential oils and a few glucosids have also considerable effect on sterilization.

(5) Sterilizing action of "wasabi" and ginger is very strong. Their sterilizing power is proportional to the degree of bitterness. Accordingly there is close correlation between sterilizing action and bitter component of material.

(6) Vinegar and sauce have very strog sterilizing power. Their chief sterilizing component are complex.

(7) About these complex sterilizing component I will study later.

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Liberation of Vitamin B$_2$ (Vitamin G) Adsorbed on Acid Earth by the Action of Pancreatin.

By

Waro NAKAHARA, Fumito INUKAI and Shu KATO.

(Reprinted from Sci. paper I. P. C. R. Vol. 24, No. 522, pp 235~334, 1934)

In contrast to the ready adsorbability of vitamin B$_2$ (=Vitamin G) by a number of adsorbents considerable difficulty was experienced by previous workers in their attempts to release the adsorbed vitamin B$_2$(1). As far as we are aware no claim has been made of a successful elution, except by Kuhn, Gyorgy and Wagner-Jauregg,(2) who used pyridin as eluent. It seems to us, however, that the results of their animal tests are not as convincing as might be desired*.

Recently we found that vitamin B$_2$ adsorbed by acid earth from liver extract can be liberated through the action of pancreatin. In the present paper we shall first give an account of our experiments on the adsorption of vitamin B$_2$ by acid earth, and then proceed to describe our method of liberating the adsorbed vitaman B$_2$.

Adsorption of Vitamin B$_2$ by Acid Earth.

The liver extract used in this experiment was prepared as follows: 1 kg of fresh beef liver was mashed and thoroughly extracted with 5 litres of 60 % alcohol containing 50 c.c. of 25% sulphuric acid. Extract was separated by filtration and sufficient sulphuric acid added to bring the reaction of the extract to pH 3~4.

To this extract was added white acid earth in the ratio of 20 g of acid earth to 1 litre of extract, and the mixture was maintained at the temperature of 30°C for 2 hours with occasional stirring. The acid earth was then recovered by filtration and was repeatedly washed with acidified water, with alcohol, and then with ether.

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* We hope to take up this question in our later experiment.

The vitamin B_2 potency of the acid earth treated in this manner was tested in accordance with the usual method. Young albino rats were first maintained on a synthetic basal diet, which was deficient in vitamin B_2, and after a period of two weeks on this diet supplementary feeding of the acid earth adsorbate was commenced and the growth response of the rats noted. The composition of the vitamin B_2 deficient diet was as follows:—

Polished rice powder	75 g
Fish protein	10 g

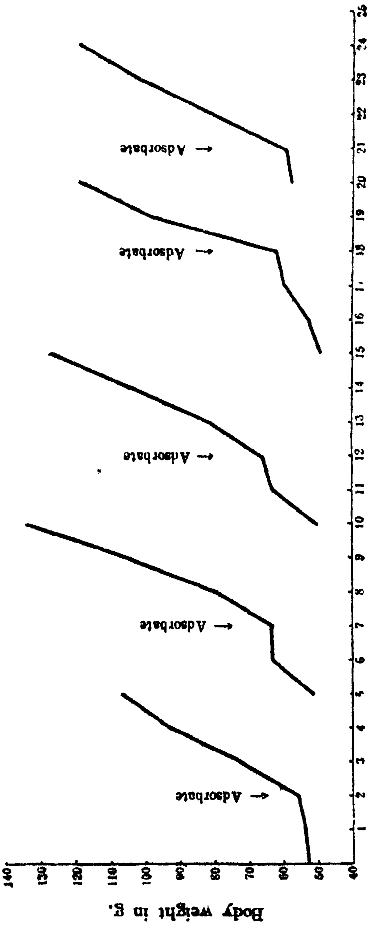

Chart 1—Growth curves of 5 rats showing the growth response to adsorbate on acid earth of liver extract ("activated" acid earth).

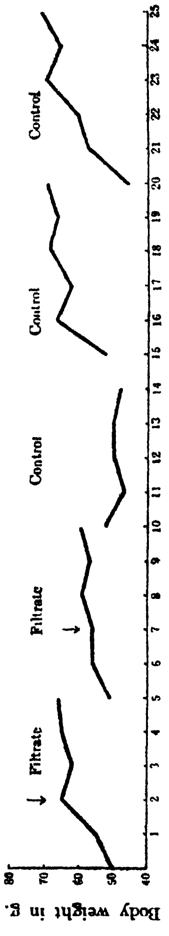

Chart 2—Growth curves of 2 rats showing the lack of vitamin B_2 action of the filtrate after the removal of adsorbate. Also included in the chart are growth curves of 3 controls on vitamin B_2 deficient diet.

Butter	10 g
McCollum's salt mixture	5 g
Oryzanin solution	5 c.c.

Chart 1 represents growth curves of 7 rats on which the effect of adsorbate was tested. In these tests 20 g of the adsorbate ("activated" acid earth) was added to 100 g of basal diet, and all the rats responded very promptly by the typical sharp increase in their body weight.

Chart 2 shows the growth curves of 2 rats on which was tested the effect of the non-adsorbable fraction after the removal of acid earth. This fraction was added to 100 g of basal diet in amounts corresponding to the yield from 1 litre of extract.

The total lack of growth response to the supplementary feeding demonstrates that the vitamin B_2 in the original extract has been entirely removed by adsorption on acid earth.

Growth curves of 3 control rats maintained on the basal diet alone throughout are also included in Chart 2.

Liberation of Adsorbed Vitamin B_2 by Pancreatin Solution.

To 1 per cent solution of commercial pancreatin preparation in 0.1 per cent aqueous solution of sodium-bicarbonate was added the "activated" acid earth in the proportion of 10 per cent, the reaction of the mixture being brought to pH 7.6 by adding sodium bicarbonate. The mixture was kept at 32°C for twenty-four hours under toluol, with frequent stirring, after which the sediment (extracted acid earth) was removed by filtration. The sediment was washed with water, and the wash water was combined with the filtrate, and the whole was concentrated into a suitable volume at a low temperature under reduced pressure in the atmosphere of CO_2 gas. The pancreatin contained in the concentrate was inactivated by heating at 85°C for 20 minutes.

The vitamin B potency of this substance, as well as of the sediment or extracted acid earth, were tested in the same manner as in the preceding experiment.

Chart 3 shows the growth curves of 6 rats on which was tested the effect of different amounts of the substance liberated by the action of pancreatin. The extraordinarily active growth response induced by the supplementary feeding of this eluated substance is remarkable, and it is obvious that the amount recoverable from 5 g of the "activated" acid earth is quite adequate as the source of vitamin B_2.

The extracted acid earth after the removal of eluate was washed repeatedly with distilled water and was tested as to its vitamin B_2 potency in order to determine the extent to which the adsorbed vitamin B_2 was liberated by the treatment with pancreatin. 20 g of the washed and dried acid earth was added to 100 g of the vitamin B$_2$ deficient basal diet.

As may be seen from Chart 4, the extracted acid earth still contained some vitamin B$_2$, showing that the liberation was by no means complete.

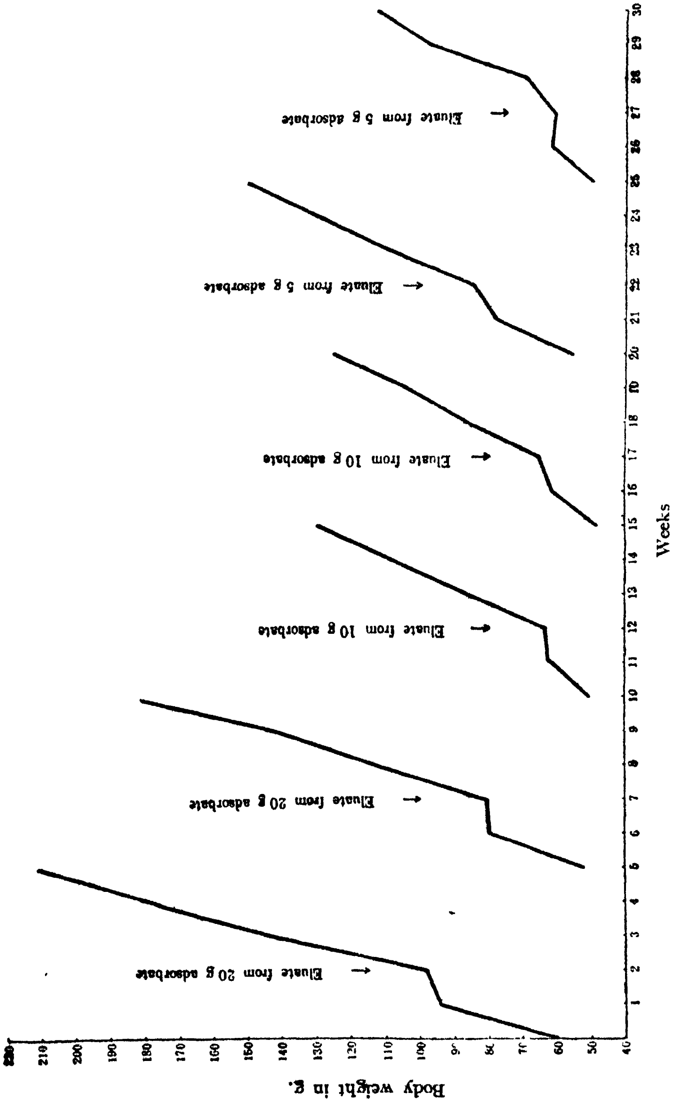

Chart 3—Growth curves of 6 rats showing the vitamin B$_2$ action of pancreatin eluate in amounts corresponding to yields from 5~20 g of activated acid earth.

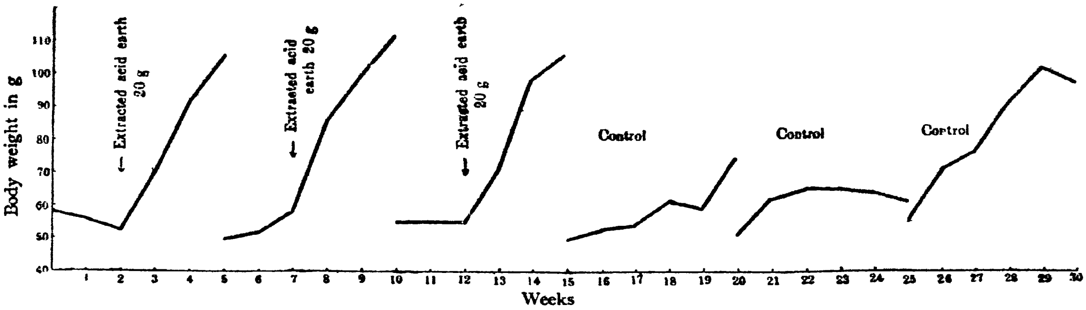

Chart 4—Growth curves of 3 rats on which was tested the effect of extracted acid earth, i.e., the sediment after the removal of the eluate. Growth curves of 3 controls are also included.

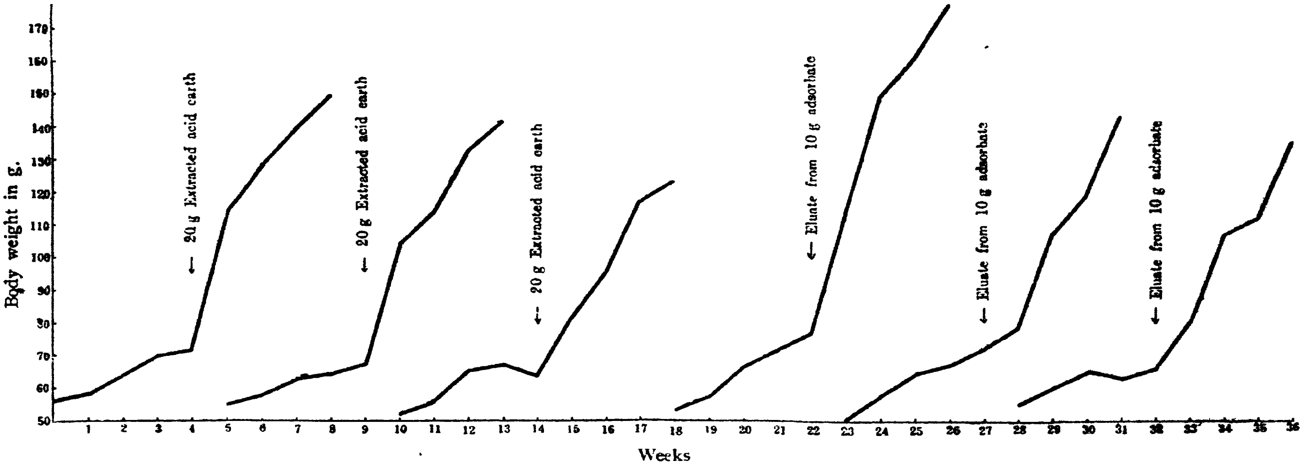

Chart 5—A comparison of the vitamin B_2 action between 20 g of acid earth after the removal of eluate ("extracted acid earth") and eluate from 10 g of activated earth.

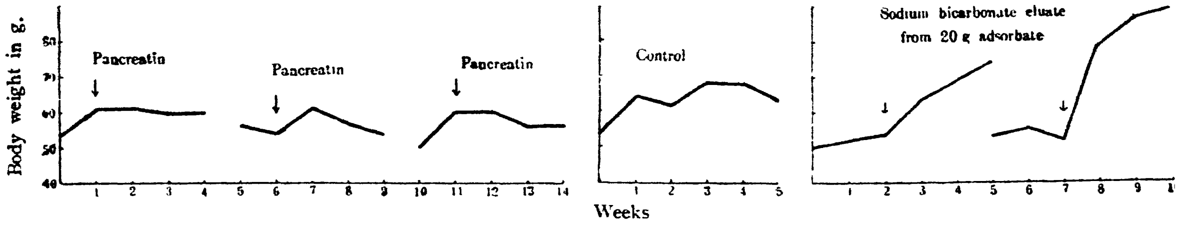

Chart 6—Growth curves of 3 rats showing the absence of vitamin B_2 action in pancreatin itself. In addition a single control on vitamin B_2 deficient diet, and growth curves of 2 rats showing a weak response to sodium bicarbonate eluate are also included.

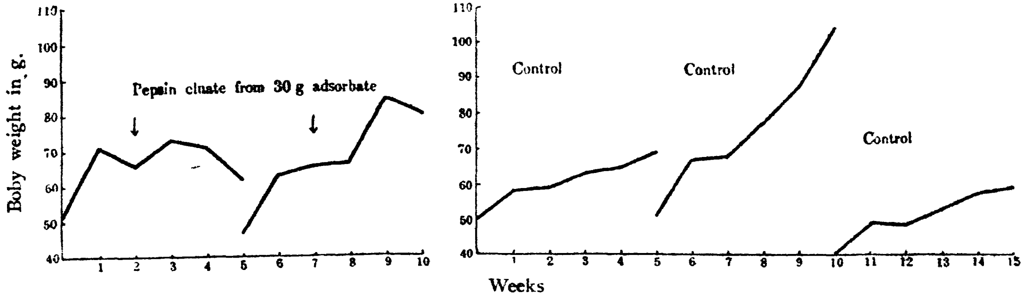

Chart 7—Growth curves of 2 rats showing the lack of growth response to pepsin eluate. Growth curves of 3 vitamin B_2 deficient controls are also included.

The growth response of the rats to a 20 g supplement of extracted acid earth was of about the same magnitude as that which follows a supplement of eluate in amounts corresponding to yields from 10 g or 5 g of "activated" acid earth.

It may be regarded as probable that more than two-thirds the total amount of adsorbed vitamin B_2 was liberated by our method using pancreatin. An additional experiment showed that this supposition is correct, since, in a parallel test, 20 g of extracted acid earth was found to be slightly less potent than the eluate from 10 g of activated earth (Chart 5).

For control to the above, tests were made also for the possible vitamin B_2 potency of pancreatin itself. 2 g of the same commercial sample of pancreatin as used in the elution experiment was added to 100 g of the basal diet. As shown in Chart 6, pancreatin itself has no growth promoting action.

Needless to say that no satisfactory elution of vitamin B_2 took place when the activated acid earth was treated with 0.1% solution of sodium bicarbonate without pancreatin, although a small amount of the vitamin seemed to dissolve out into the solution. See Chart 6.

The possibility of the elution of adsorbed vitamin B_2 by pepsin solution was also examined in passing. The procedure of the manipulation was the same as in the case of pancreatin solution, which was simply replaced by one per cent solution of commercial pepsin in 0.15 per cent hydrochloric acid. As may be seen from Chart 7, no satisfactory liberation of vitamin B_2 was attained by the action of pepsin solution.

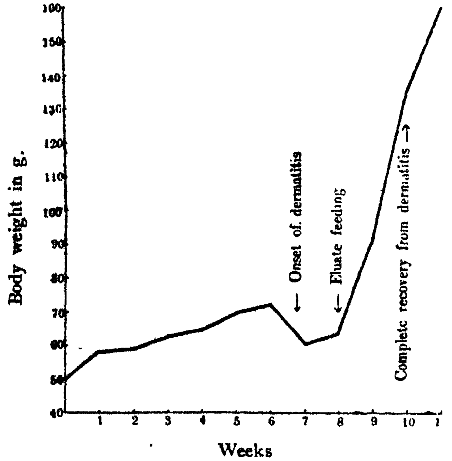

Chart 8—Growth curve of the rat developing pellagra-like symptoms on vitamin B_2 deficient diet, showing the curative effect of our eluated vitamin B_2.

Curative Action of Eluate on the so-called Pellagra-like Condition.

It is well known that vitamin B_2 deficiency produces in certain proportions of the rats pathological conditions somewhat similar to pellagra in man, and that a timely administration of vitamin B_2 brings about the disappearance of these symptoms.

Among our series of vitamin B_2 deficient rats we happened to encounter a single case with definitely recognizable symptoms and we had the opportunity of testing the curative action of our eluated vitamin B_2.

Chart 8 gives the growth curve

of the rat in question, which was fed on the same vitamin B_2 deficient diet as before. It developed marked pellagra-like symptoms during the seventh week and the end of the eighth week we began adding our eluate, in amounts corresponding to the yield from 20 g of activated acid earth, to 100 g of the basal diet. The growth response of the rat was typical.

Figure 1 illustrates the condition of the rat one day before the beginning of the vitamin B_2 supplement, showing dermatitis over the chest and under the arm, and also alopecia around the eye.

Figure 2 represents the same rat 14 days after the beginning of vitamin B_2 supplement, showing a complete recovery from dermatitis.

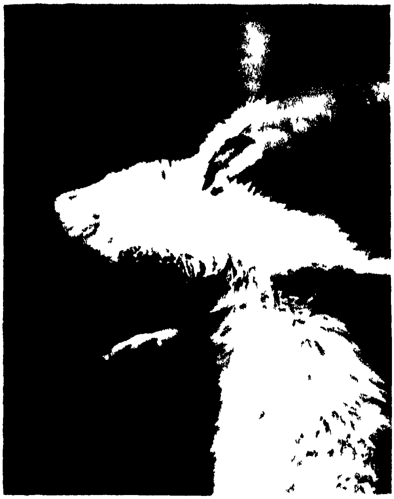

Figure 1—Rat A, 1 day before the commencement of eluted vitamin B feeding. Note the extreme mal nutrition and typical dermatitis

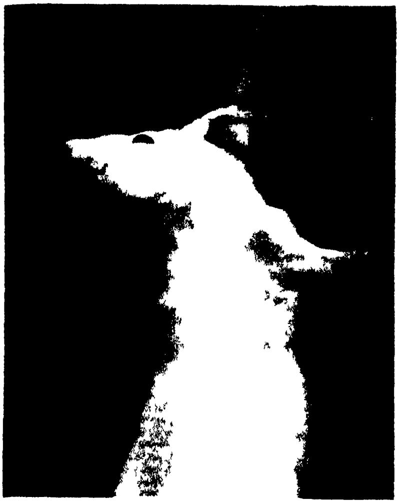

Figure 2—The same rat, after 14 days of eluated vitamin B_2 feeding, showing a complete disappearance of dermatitis,

Comments.

From the physiological point of view, inasmuch as the vitamin B_2 adsorbed on acid earth is utilized by the animal, it is self-evident that the elution of vitamin B_2 must take place somewhere in the alimentary canal. Our present experiments show that this elution must occur in the intestine rather than in the stomach, since the elution takes place *in vitro* under the influeces of pancreatin, but not through the action of pepsin.

Another point of interest in our experimental results is that vitamin B_2

is much better utilized by the animal when supplied in the form of eluate than when given adsorbed on acid earth. This difference in the rate of utilization suggests that no small amount of vitamin B_2 is excreted along with the acid earth which carries it. Our method of liberating vitamin B_2 from the adsorbent therefore offers the advantage, not only of doing away with the ingestion of bulky adsorbent, but also of facilitating the utilization by the animals of the vitamin B_2 administered to them.

Conclution.

Vitamin B_2 in liver extract is completely absorbable by acid earth, but from the experience of previous workers, it may be expected to be difficult to eluate it by ordinary means.

In the foregoing experiments we found that the adsorbed vitamin B_2 can be satisfactorily released by the action of pancreatin. Moreover, it was found that the vitamin B_2 thus released is more readily utilized by the animal organism than the same vitamin adsorbed on acid earth.

Our cordial thanks are due to Professer U. Suzuki, M. I. A , for his kind advice and helpful criticisms given us in the course of this work.

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Isolation of Vitamin C from Vegetables and the Relations between Vitamin C and Ascorbic Acid*.

By

Suttekiti MARUYAMA.

(Received September 19, 1934.)

In recent years no chapter but that of C in the vitamin research has made so wonderful a development.

Szent-György(1) first isolated in 1928 from suprarenal cortex a reducing crystalline substance consisting of an elementary composition of $C_6H_8O_6$, but, as it seemd to be an isomer of glycuronic acid, he called it "Hexuronic Acid". This compound was obtained by him also from orange and cabbage.

Tillmans and his collaborators(2), noticing the properties of hexuronic acid reported by Szent-Gyorgyi, made a supposition in 1932 that Szent-Gyorgyi's hexuronic acid might be identical with vitamin C. This, in fact, was based upon their own theory that both the reducing capacity and vitamin-C activity in vegetables were always parallel.

Almost simultaneously and independently, Szent-Gyorgyi(3) made a remarkable observation that his hexuronic acid could prevent or cure experimental scurvy in guinea pigs by giveng them *per os* a daily dose of 1 mg of the acid. He then claimed that hexuronic acid would most probably be identical with vitamin C

This finding by Szent-Gyorgyi was traced and confirmed by many other workers who showed that hexuronic acid obtained not only from suprarenal cortex, but also from such different vegetable sources, as lemon juice(4), hawthorn(5), and a Hungarian red pepper(6), had possessed nearly the same antiscorbutic activity.

This active compound is now called "Ascorbic Acid" by Szent-Gyorgyi.

With regard to the question whether or not ascorbic acid and vitamin C are identical, there are valuable works as follows: Demole(7) stated that four different compounds though mutually related, namely ascorbic acid, its sodium salt, its reversible oxidation product (dehydro-ascorbic acid) and ascorbic acid regenerated from the latter, (the first two had been recrystallized several times) had nearly the same antiscorbutic activity in a daily allowance of 1 mg. Micheel and Moll(8) made an excellent demonstration showing that monoacetone derivative of ascorbic acid, when administrated subcutaneously, was completely inactive, in spite of the fact that ascorbic acid regenerated from it by saponification, indicated the same activity as the initial ascorbic acid, in oral as well as in subcutaneous administration. Svirbely and Szent-Gyorgyi(6) pointed out that the said acetone derivative of ascorbic acid, in oral administration, was from 1/3 to 1/2 as active as ascorbic acid itself. More recently, T. Reichstein(9) announced that his synthesized ascorbic acid was as active as natural ascorbic acid. This evidence may be a decisive one in favour of the identity of vitamin C with ascorbic acid. However, the data concerning animal experiments are not as yet reported.

This was the situation in the research of vitamin C at the beginning of the present work.

In Japan, however, ascorbic acid, so far as the author knows, has not been isolated in crystalline form, to say nothing of testing its anti-scorbutic effect. This led him to undertake experiment along with this line of research.

First, an attempt was made to obtain ascorbic acid from suprarenal cortex as starting material, by following as strictly as possible the procedure recommended by Szent-Györgyi⁽¹⁾, but in vain.

This failure on the part of the author may have been due to his unskillfullness. The procedure, as then modified by him, finally gave satisfactory results; a reducing crystalline substance so obtained, perfectly coincided in chemical and physical properties with ascorbic acid reported by Szent-Györgyi or by Tillmans.

This first experience led him to study further as to whether ascorbic acid could be found in different kinds of native vegatables already known to contain vitamin C, such as "Daikon" juice(10) (Daikon, *Rhapanus Sativus* L.), "Natsumikan" juice(10) (a kind of Japanese lemon, *Citrus Aurantium*, L.) and Japanese green tea.(11) In fact, one and the same ascorbic acid could be obtained in crystalline form from all these materials by means of his own isolation method.

The first two kinds af ascorbic acid preparations, namely, preparation from suprarenal cortex of oxen and that from Daikon juice, were examined for their antiscorbutic activity on guinea pigs. The result showed that both preparations were equally effective by feeding them with a daily dose of 0.8 mg of the preparation.

After all these findings, the author has come to think that ascorbic acid taken from the other materials is also effective.

An animal experiment is now in progress to verify this possibility. So far as the results hitherto obtained are concerned, ascorbic acid preparation from Japanese green tea is found to be as effective as those from the other two materials. 4 guinea pigs that have been fed on both vitamin-C-deficient basal diet and ascorbic acid obtained from Japanese green tea as supplement to the basal diet, have survived for 5 weeks from the beginning of administrating the acid, while four negative control animals fed on the basal diet alone, died of severe scurvy within the first several days. Those surviving animals are now gradually increasing their weight and are recovering from scurvy.

As soon as the animal experiment employing ascorbic acid taken from Japanese green tea, reveals a satisfactory development, the author expects to report the results later on, more in detail.

Experimental Part.

*Isolation of Ascorbic Acid from Suprarenal Cortex of Oxen.**

The author has succeeded in obtaining ascorbic acid in a satisfactory manner as given below:—

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* It is needless to say that in isolating ascorbic acid from natural stuffs, the author in any case carried out all of the necessary operations in an atmosphere of carbon dioxide as much as possible and at a temperature as low as possible. In the course of this and other isolation experiments described in the latter part of this paper, ascorbic acid existing was quantitatively determined from time to time by Tillmans' titration method with dichlorphenol-indophenol, in order to justify any operations tried. (refer to: J. Tillmans: loc. cit.)

2,100 c.c. of methyl alcohol mixed with 0.21 c.c. of 10 per cent aqueous solution of sodium cyanide, was bubbled, while being cooled in ice water, from carbon dioxide for half an hour. To this cold mixture was then added 700 g of fresh suprarenal cortex freshly cut into small pieces with a meat chopper, and a hot saturated aqueous solution of 11 g of barium acetate. The whole mixture was then cooled and bubbled for another half an hour. The mixture was then pressed out through cotton cloth.

The turbid filtrate was clarified through filter paper, and to the filtrate was added a hot saturated aqueous solution of 110 g of lead acetate all at once, and then cooling and bubbling were continued for an hour. The lead salt which separated was collected on a suction funnel and was carefully pressed and washed with two small portions of cold methyl alcohol to which were added a few drops of the above-mentioned lead acetate solution. The lead salt was ground down, placed into a little amount of cold water and promptly decomposed by adding 20 per cent sulphuric acid in a slight excess

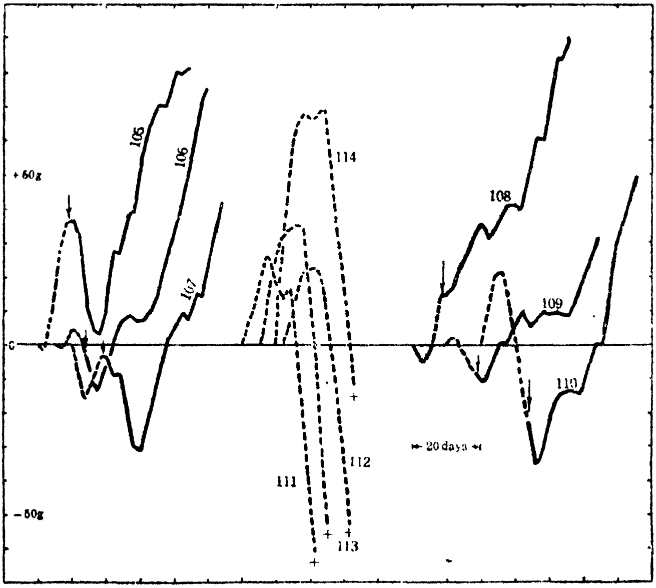

Chart 1—Growth-curves of guinea pigs fed on vitamin-C deficient diet with and without ascorbic acid supplement. Animals No. 105—107 took daily dose of 0.8 mg of the ascorbic acid preparation from suprarenal cortex; No. 108—110, daily dose of 0.8 mg of the preparation from Daikon juice. ------------: basal diet alone, ————: basal diet and supplement.

until a test sample of the mixture distinctly became acid against thymol blue paper. Then the precipitate of lead salt was separated with a centrifuge and was washed twice with small portions of cold water.

The combined clear solutions were then evaporated in vacuum at a temperature not higher than 30°, to be dry. The dry residue was extracted by methyl alcohol, which dissolved nearly all of the residue but the inorganic salts. These salts were separated by filtration and washed with methyl alcohol.

The methyl alcoholic filtrate was again concentrated under rejuice and melting at 178—180°.

10 guinea pigs were fed on the basal diet deficient of vitamin C. The first symptoms experimental scurvy were observed within about 10 days:—abnormal liveliness or passive movement, sensibility to pressure, interruption of growth or decrease of weight, etc.

On the 9th day they were divided into 3 groups, of which the first included 3 animals, No. 105, 106 and 107, the second 3 animals, No. 108, 109 and 110, and the 3rd 4 animals, No. 111, 112, 113 and 114. In the 1st group each one was administrated every day *per os* with 0.8 mg of ascorbic acid preparation from suprarenal cortex, in the 2nd with 0.8 mg of that from Daikon juice and in the 3rd, as negative control, with no such preparation as supplement to the basal diet. The beginning of the administration of the test substances in the chart is indicated with a small arrow. All animals in the 1st group and in the 2nd group soon restored their usual health and normal growth. At the end of experiment, which, owing to the lack of test material, continued only for 45 days, they showed their complete healthiness. They were then chloroformed and subjected to autopsy. The section, in fact, revealed no symptom of scurvy, only indicating a trace of swell on the joints between bones and cartilages of ribs. So, if the process of feeding had been kept on, this trace would have also been cleared off.

The 4 animals in the 3rd group, on the contrary, gradually decreased their weight, and soon died. In the autopsy severe symptoms of experimental scurvy were observed as: swollen condition in the boundaries of bones and cartilages of ribs, swollen condition in region of knee-joint, subcutaneous and intramusculary hemorrhages, etc.

From the foregoing result of therapeutic experiments there is no doubt that so far as ascorbic acid from suprarenal cortex of oxen and ascorbic acid from Daikon juice are concerned, they were anti-scorbutically effective on experimental scurvy in guinea pigs in a daily dose of 0.8 mg.

Ascorbic acid preparations thus tested may be as yet somewhat impure, when it is considered that their melting point is lower than that reported on

Photo 1

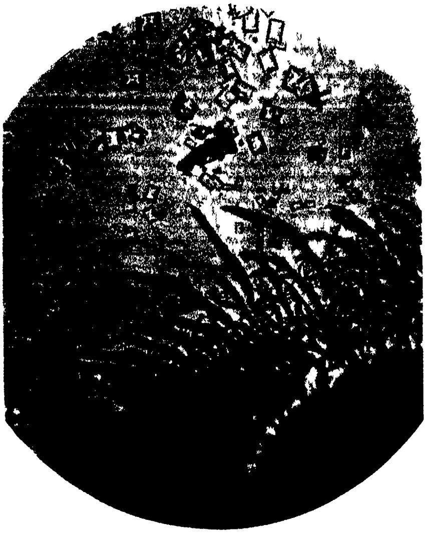

Photo 2

Photo 1 and 2—Ascorbic acid from suprarenal cortex, recrystd from acetone hexane

Photo 3

Photo 4

Photo 3 and 4—Ascorbic acid from Daikon juice, recrystd from acetone dioxane and from acetone ether-petroleum-ether respectively

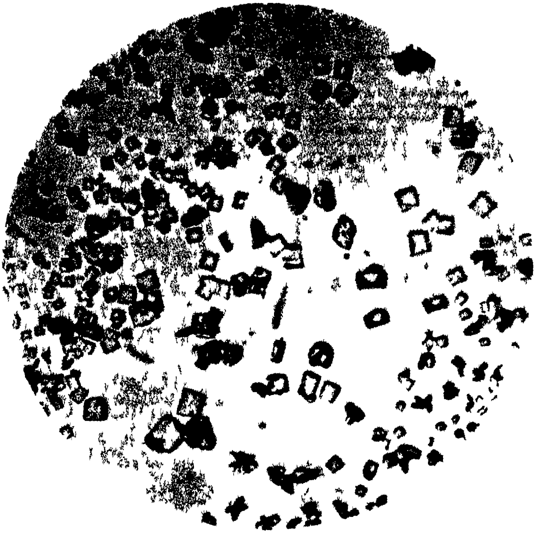

Photo 5—Ascorbic acid from Natsumikan juice crystals from the 1st crystallization from acetone-ether petroleum ether

Photo 6

Photo 7

Photo 6 and 7—Ascorbic acid from Japanese green tea recrystd from acetone-dioxane purest ascorbic acid, and, therefore, it may be possible that the preparations throughly purified might be effective even in smaller quantity of dose.

Summary.

Various statements made by chemists relating to vitamin C have briefly been mentioned in order to show how the author developed its research commenced early in Feb. 1934.

Ascorbic acid has been isolated in crystalline form not only from suprarenal cortex of oxen, but also from Daikon juice (Daikon, *Rhapanus Sativus*. L.), Natumikan juice (a kind of Japanese lemon, *Citrus Aurantium*, L. *var. sinensis*, Engl.) and Japanese green tea. By its elementary analytical values, specific rotation and other chemical and physical properties, this ascorbic acid has been proved to be identical with the existing acid.

Two therapeutic experiments were carried out with positive results parallel to each other, to examine the first two ascorbic acid preparations, namely, preparation obtained from suprarenal cortex of oxen and that from Daikon juice, for antiscorbutic activity on scurvy in guinea pigs, and the results show, so far as these preparations are concerned, that this ascorbic acid was effective in a daily dose of 0.8 mg.

Another therapeutic experiment using an ascorbic acid preparation obtained from Japanese green tea, is now in progress. So far this preparation seems to be effective also.

These evidences seem to support Szent-Gyorgyi's theory that vitamin C and ascorbic acid are identical.

A slight but favourable modification has been made on the method of Szent-Gyorgyi for obtaining ascorbic acid from suprarenal cortex.

A full description of procedure for isolating ascorbic acid is given in the case of suprarenal cortex and of Daikon juice.

In connection with the case of Natumikan juice or Japanese green tea, where isolation procedure was substantially the same as that in the other cases, are given some notes necessary for isolating ascorbic acid.

Dichlorphenol-indophenol—a reagent proposed by Tillmans as one capable of determining vitamin C in foods—has been applied in the case of suprarenal cortex and of the other vegetables in question, and, in fact, turned out to play such a rôle.

This fact confirms Tillmans' view that the vitamin C and reducing factor in vegetables are always parallel.

This work has been carried out under the supervision of Professer Umetaro Suzuki, to whom the author greatly owes. The author feels also grateful toward D. Saburo Hunahasi who accomplished all the elementary analysis.

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Kationenumtausch an den Tonen. (I. Mitteilung)

Von

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(Eingegagen am 12, September 1934)

Zur Aufklärung der Beziehung von Kationenumtausch und Struktur bei den Tonen, hat der Verfasser vier Sorten der Tonen, d. h., Bentonit, Hettenleidelheimer Fettton, Saarauer Blauton und Zettlitzer Kaolin, deren Eigenschaften von einander sehr verschieden sind, als die Versuchsmaterialien gebraucht. Sie wurden zu erst als Calciumton geändert. Dann wurden die ausgetauschte Ca-Menge gegen verschiedene Aminen mit verschiedenen Ionengrössen bestimmt. Die Ionengrösse der einzutauschender Kationen haben auf die ausgetauschte Menge des Calciums keine Einflüsse ausgeübt. Daraus hat der Verfasser so zusammengefasst, dass der Kationenumtausch an den Tonen nur auf der Oberfläche stattfinden soll.

Die Werte, die nach der Freundlichschen Formel berechnet wurden, haben mit den experimentellen werten sehr gut übereinstimmt.

Biochemical Studies on "Sotetsu", the Japanese Sago Plant. II.

On the Chemical Constituents, especially the Sex Differences of "Sotetsu"-stems.

By

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(Received September 26, 1934.)

The places of production of the "sotetsu"-stems experimented were at Ōshima and Sata in Kagoshina prefecture; and the kinds of the samples obtained from these districts, respectively were as follows:

(1) Female stem A, which was born in 1933, and not born in 1932.
(2) Female stem B, which was born in 1932, and not born in 1933.
(3) Male stem.

These "sotetsu"-stems were studied on the both sides of layer- and sex-differences, in January, 1934.

The "sotetsu"-stem is consist of four layers that is (1) pith, (2) xylem or wood part, (3) cortex and (4) scaly part, and the ratio by weight of these parts is 10 : 20 : 30 : 40 roughly.

The "sotetsu"-stem, especially the pith and the cortex contains large amount of starch; therefore sometimes used for starch manufacture.

Experimental Results.

I. Chemical Composition of Pith and Cortex.

The chemical composition of the "sotetsu"-stem from Ōshima, was estimated as following table:

	Female stems				Male stem	
	A (born in 1933)		B (born in 1932)			
	Pith	Cortex	Pith	Cortex	Pith	Cortex
Water	81.92	78.26	75.65	78.85	83.96	78.09
Crude protein	2.11	1.70	1.51	1.69	0.81	1.52
Protein	1.09	1.48	0.88	1.30	0.67	0.81
Crude fat	0 08	0.12	0.07	0.11	0.05	0.10
Crude fibre	1.41	2.98	1.52	2.43	1.08	2.31
Crude ash	0.62	0.76	1.02	0.94	0.50	0.71

N-free extract	13.88	16.18	20.23	15.98	13.60	17.27
Total carbohydrate	12.76	13.55	18.40	13.23	12.17	14.93
Starch	5.37	5.19	9.30	6.40	6.02	8.78
Dextrin	0.78	0.87	2.74	1.60	0.56	0.65
Reducing sugar (as glucose)	2.53	3.94	1.63	2.23	1.54	2.27
Non-reducing sugar (as sucrose)	3.22	2.73	3.22	2.00	3.16	2.07

II. Sex Differences of Stem-composition.

The pith and cortex of "sotetsu"-stems obtained from Ōshima and Sata, was analysed respectively in both the male and female stems. The results were as follows:

	Samples, obtained from Ōshima						Samples, obtained from Sata		
	Pith			Cortex			Pith		
	Male	Female A born in 1932	Female B born in 1933	Male	Female A born in 1932	Female B born in 1933	Male	Female A born in 1932	Female B born in 1933
Water (%)	84.96	75.65	81.92	78.09	78.85	78.26	70.40	69.52	78.74
Dry matter	16.04	24 35	18.08	21.91	21.15	21.74	29.60	30.48	21.26
	In 100 parts of dry matter								
Crude protein	5.02	6.18	11.65	6.96	8.00	7.84	5.48	8.88	7.40
Protein	4.14	3.63	6.04	3.68	6.16	6.80	4.09	5.24	6.17
Crude fat	0.31	0.27	0.43	0.47	0.52	0.54	0.33	0.17	0.55
Crude fibre	6.72	6.23	7.78	10.53	11.51	13.71	4.48	3.65	9.82
Crude ash	3.08	4.17	3.45	3.26	4.44	3.51	3.27	3.63	4.37
N-free extract	84.87	83.15	70.55	78.78	75.53	74.40	86.44	83.67	77.86
Starch	37.52	38.21	29.71	40.08	30.22	23.88	56.14	54.96	30.43
Dextrin	3.50	11.23	4.30	2.97	7.59	4.02	3.06	6.97	1.39
Reducing sugar (as glucose)	9.58	6.70	14.00	10.35	10.55	18.12	5.25	4.08	10.22
Non-reducing sugar (as sucrose)	19.67	13.23	17.83	9.44	9.43	12.56	10.02	7.17	17.63

III. Peroxidase action of "Sotetsu"-stem.

The peroxidase action of "sotetsu"-stems was compared with layer differences and sex differences respectively, by means of the colourimetric guaiacum tincture method.

(a) Comparison of peroxidase action of pith and cortex.

	Male stem		Female stem, A (born in 1933)		Female stem, B	
	Pith	Cortex	Pith	Cortex	Pith	Cortex
Colorimeter reading.	10 mm	5.5 mm	10 mm	5.8 mm	10 mm	4.8 mm
Ratio of cortex to 100 of pith.	100	182	100	172	100	208

(b) Comparison of peroxidase action of male- and female-stems.

	Pith			Cortex		
	Male	Female A (born in 1933)	Female B (born in 1932)	Male	Female A (born in 1933)	Female B (born in 1932)
Colorimeter reading.	10.0 mm	11 2 mm	8.5 mm	10.0 mm	15.0 mm	10.0 mm
Ratio of female to 100 of male.	100	89	118	100	67	100

Summary.

From the results of the above experiments we can summarize the following differences in chemical properties between the pith and cortex of "sotetsu"-plant.

(1) The starch content of the pith is greater than that of the cortex but in the case of male pant shows a reversed tendency.

(2) The amount of reducing sugar of the pith is less than that of the cortex. On the other hand, the non-reducing sugar content of the pith is always superior to that of the cortex.

(3) The crude fat and fibre contents of the pith are always inferior to those of the cortex.

(4) A very significant difference of the peroxidase action was observed between the pith- and cortex-juice; that is, in the juice of cortex, the enzyme action is more active .than that of the pith.

Concerning the sex differences on the chemical properties of "sotetsu"-plant, we can summarize as follows:

(5) Difference of the quantity of starch between the male and female (A and B) plants were determined. In those results, there is nothing to choose between the two stems of male and female B; then in the female A, considerably small amount of starch than those of the male and female B, was estimated. This phenomena shows that the starch was consumed newly, in order to bear the seed.

(6) The dextrin content of the female B is very large than those of other stems.

(7) The content of reducing sugar of the female A was considerably greater than those of other stems. The same tendency was observed in the amount of non-reducing sugar.

(8) The protein and ash contents of the female stems in both A and B are always greater than those of male stem.

(9) The peroxidase action of the juice of female stem B is almost equal to that of male stem, but the enzyme action of female stem A is poor.